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January 21, 2009

Dear Sir/Madame,

GRAS Exemption Claim

Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(~)(11) 62 FR 18938 (17 April 1997)]

Sweet Green Fields, LLC has determined Rebiana (rebaudioside A) to be Generally Recognized As Safe (GRAS), consistent with Section 201(s) of the Federal Food, Drug, and Cosmetic Act. This determination is based on scientific procedures as described in the following sections, under the conditions of its intended use in food. Therefore, the use of Rebiana in food, as described below, is exempt from the requirement of premarket approval.

Availability of Information

The data and information that serve as the basis for this GRAS Notification will be sent to the U.S. Food and Drug Administration (FDA) upon request, or will be available for review and copying at reasonable times at the offices of:

Dr Gary Williams
BSB, Rm. 413
New York Medical College
Valhalla, NY 10595

Warm regards

James McMurtry
CFO/Regulatory Affairs

jmcmurtry@sweetgreenfields.com

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January 21, 2009

Office of Premarket Approval (HFS-200)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C St. SW.,
Washington, DC 20204

Dear Dr Paulette Gaynor

Enclosed is a notice of a GRAS exemption claim for Rebiana (Rebaudioside A) derived from the Stevia rebaudiana, in triplicate.

Warm regards

James McMurtry
CFO/Regulatory Affairs

jmcmurtry@sweetgreenfields.com

Notice to the U.S. Food and Drug Administration (FDA) that the use of Rebiana (Rebaudioside A) derived from *Stevia rebaudiana*, as a Food Ingredient is Generally Recognized as Safe (GRAS)

Submitted by:

Sweet Green Fields, LLC
4164 Meridian Street, Suite 304
Bellingham, WA 98226, USA

Prepared by:

New York Medical College
Department of Pathology
Chemical Safety Laboratory
Valhalla, NY 10595, USA

January 15, 2009

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List of Abbreviations

%	percent
>	greater than
≥	greater than or equal to
°C	degrees Celsius
^{14}C	carbon 14 (isotope)
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism, and excretion
ALT	alanine transaminase
AUC	area under the curve
BUN	blood urea nitrogen
CAS	Chemical Abstracts Service
CFR	Code of Federal Regulations
CFU	colony-forming units
C_{max}	maximum concentration
EC	Enzyme Commission
EPA	United States (U.S.) Environmental Protection Agency
ETOH	ethanol
F	female
F_0	parental generation
F_1	first-generation offspring
F_2	second-generation offspring
FAO	Food and Agriculture Organization of the United Nations
FCC	Food and Chemical Codex
FDA	United States (U.S.) Food and Drug Administration
g	gram
GGT	gamma-glutamyl transferase
Glc	glucose
GMP	Good Manufacturing Practices
GRAS	generally recognized as safe
h	hour
HPLC	high-performance liquid chromatography
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kg	kilogram
LC-DAD-MS	liquid chromatography-diode array detection-mass spectrometry
LD_{50}	median lethal dose

LOQ	limit of quantification
M	male
mg	milligram
ml	milliliter
ng	nanogram
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
ppm	parts per million
Rha	ramnose
vs.	versus
w/v	weight/volume percentage solution
w/w	weight/weight percentage solution
WHO	World Health Organization
Xyl	xylose

EXECUTIVE SUMMARY

The subject of the present GRAS Notice is Rebiana (rebaudioside A), a steviol glycoside derived from the *Stevia rebaudiana* (Bertoni) plant. Rebiana is manufactured by Sweet Green Fields, LLC (4164 Meridian Street, Suite 304, Bellingham WA 98226, USA) under current Good Manufacturing Practices (GMP) to meet standardized specifications. Rebiana is intended to be used as a sweetener in a variety of food products in the United States, such as cereals and energy bars, and beverages such as diet soft drinks, fruit juice drinks, and iced teas at levels consistent with the acceptable daily intake (ADI) of 0-4 mg/kg body weight/day of steviol glycosides (as steviol equivalents) established in 2008 by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and Food Standards Australia New Zealand (FSANZ).

The determination that the use of Rebiana as specified is GRAS was made through scientific procedures, based on several published reports that indicate there is reasonable certainty that Rebiana is not harmful under the intended conditions of use and is therefore exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act (FDA, 1997). This determination was corroborated by a panel of qualified experts under the aegis of New York Medical College.

Text Table. Panel of qualified experts

Fisher, Gerald, PhD, Malvern, PA, USA
Pi-Sunyer, Xavier, MD, NYC, NY, USA
Williams, Gary, MD (Chairman), Valhalla, NY, USA
Iatropoulos, MD, PhD (Rapporteur), Valhalla, NY, USA

The safety of steviol glycosides (*i.e.*, rebaudioside A, stevioside) has been evaluated by various national and international authorities, including the U.S. Food and Drug Administration (FDA), JECFA, and FSANZ. Previously, JECFA requested additional information for steviol glycosides. Among the issues raised was a lack of precise material specifications, questions regarding possible genotoxic effects, and uncertainty about effects on hemodynamics and glucose homeostasis in humans. Also unresolved were questions regarding possible adverse effects on the reproduction and/or fertility, based on older published rat studies employing crude *Stevia* extracts. Several recently published studies employing highly purified steviol glycosides allowed resolution of these issues. Nonclinical studies showed no evidence of genotoxic potential or adverse effects on reproduction and offspring development. Published human studies showed no effects on blood pressure or glucose homeostasis.

In addition to safety issues, questions by JECFA regarding species- and/or compound-related differences in the metabolism of steviol glycosides have been raised. Results of comparative metabolism studies indicate that stevioside and rebaudioside A, the main glycosides in the *Stevia rebaudiana* plant, are metabolized to steviol in a similar manner in both rats and humans. Thus, stevioside and rebaudioside A safety data are mutually supportive, since they are primarily the result of steviol exposure.

Based on published weight-of-evidence safety and metabolism data the Panel concluded that Rebiana use is safe, and as such does not pose any human safety risk.

I. GRAS Exemption Claim

A. Notifier

As representative of Sweet Green Fields, LLC (4164 Meridian Street, Suite 304, Bellingham WA 98226, USA), I hereby notify the U.S. Food and Drug Administration that the use of Rebiana (rebaudioside A) in foods for the general population is generally recognized as safe (GRAS) and is therefore exempt from the premarket approval requirements of the Federal Food, Drug, and Cosmetic Act.

_____	01/15/2009
Signature	Date

Name (print): Mr. James McMurtry

Title: CFO/Regulatory Affairs

Address: 4164 Meridian Street, Suite 304, Bellingham, WA 98226

Phone: (360) 483 4555

E-mail: jmcmurtry@sweetgreenfields.com

B. Name of GRAS Substance

The subject of the present GRAS Notice is Rebiana (rebaudioside A), a steviol glycoside derived from the *Stevia rebaudiana* (Bertoni) plant.

C. Conditions of Use

Rebiana will be used as a food ingredient in a variety of food products in the United States. Rebiana would be used as a sweetener in foods such as cereals and energy bars, and beverages such as diet soft drinks, fruit juice drinks, and iced teas at levels consistent with the ADI of 0-4 mg/kg body weight/day (as steviol equivalents) established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for steviol glycosides (WHO, 2008).

D. Basis for GRAS determination

The determination that the use of Rebiana in foods for the general population is GRAS was made through scientific procedures. The technical evidence of safety, most of which appears in the published scientific literature, is considered by the notifier to be sufficient in quantity and quality to provide reasonable certainty that Rebiana is not harmful under the intended conditions of use. Moreover, Rebiana use is safe, and as such does not pose any human safety risk. This determination was made by a panel of qualified experts.

The present GRAS Notification summarizes information pertaining to the safety of Rebiana. Published data were obtained through searches of various scientific and toxicological databases.

In accordance with Volume 62, Number 74 of the April 17, 1997 Federal Register, Pages 18937 to 18964 (Proposed Rules, 21 CFR Part 170), the present document addresses the following key elements:

IDENTITY AND SPECIFICATIONS

Common and chemical name;

Chemical Abstracts Service (CAS) registry number;

Enzyme Commission (EC) number;

Empirical and structural formula;

Quantitative composition;

Method of manufacture (excluding any trade secret information);

Characteristic properties;

Any potential human toxicants;

Specifications for food-grade material (may relate to identity, purity, or both).

CONDITIONS OF USE

Foods in which the substance is to be used;

Levels of use, including self-limiting levels;

Purposes for which the substance is used, including, when appropriate, a description of the population expected to consume the substance.

TECHNICAL EVIDENCE OF SAFETY

A comprehensive discussion of, and citations to, generally available and accepted scientific data, information, methods, or principles that the notifier relies on to establish safety, such as:

Summaries of any applicable toxicological studies describing the studies, conclusions, and relevance to the GRAS determination;

Consideration of the probable consumption of the substance and the cumulative effect of the substance in the diet;

Comprehensive discussion of any reports of investigations or other information (e.g., adverse event reports and consumer complaints) that may appear to be inconsistent with a GRAS determination.

II. Identity and Specifications

A. Product Characterization

Common Name(s): Rebaudioside A (≥97% purity); Rebiana

Chemical Name: 19-O-beta-glucopyranosyl-13-O-(beta-glucopyranosyl(1-2)-beta-glucopyranosyl(1-3))-beta-glucopyranosyl-13-hydroxykaur-16-en-19-oic acid

CAS Registry No.: 58543-16-1

Molecular weight: 967.014

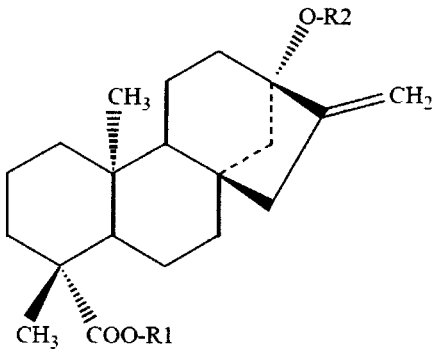
Rebaudioside A (≥97% purity) (Rebiana henceforth) is a glycoside derived from the plant *Stevia rebaudiana* Bertoni. The *Stevia* plant contains a complex mixture of naturally-sweet diterpene glycosides (steviol glycosides), and extracts of *Stevia*, primarily the leaves, have been used in South America and Asia as sweeteners. Rebaudioside A is considered to be the sweetest glycoside, with the least bitter aftertaste. The Stevia plant has been consumed for many years in Japan and by indigenous peoples in Paraguay (Carakostas *et al.*, 2008).

To date, Sweet Green Fields, LLC has not received any adverse event reports or consumer complaints regarding the failure of its Rebiana product to meet specifications or be construed as a health and safety concern that would in any way adversely affect or be inconsistent with this GRAS determination.

Stevia is reported to contain 52.8% total carbohydrates, 15% stevioside, 6.2% protein, 5.6% lipids; the remainder (20.4%) is comprised of water-soluble substances, di- and triterpenes, sterols, flavonoids, volatile oils, pigments, gums, and inorganic matter (Kinghorn, 2002). The

glycosides derived from *Stevia* (steviol glycosides) are: stevioside (4-13% dry weight), rebaudiosides A (2-4%) and C (1-2%), dulcoside A (0.4-0.7%), and traces of steviolbioside and rebaudiosides B, D, and E (Kinghorn, 2002).

Figure 1. Structure of glycosides derived from *Stevia rebaudiana*



Compound Name	R1	R2
steviol	H	H
steviolbioside	H	β-Glc-β-Glc(2→1) [a]
stevioside	β-Glc	β-Glc-β-Glc(2→1)
rebaudioside A	β-Glc	β-Glc-β-Glc(2→1) | β-Glc(3→1)
rebaudioside B	H	β-Glc-β-Glc(2→1) | β-Glc(3→1)
rebaudioside C (dulcoside B)	β-Glc	β-Glc-α-Rha(2→1) | β-Glc(3→1)
rebaudioside D	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1) | β-Glc(3→1)
rebaudioside E	β-Glc-β-Glc(2→1)	β-Glc-β-Glc(2→1)
rebaudioside F	β-Glc	β-Glc-β-Xyl(2→1) | β-Glc(3→1)
dulcoside A	β-Glc	β-Glc-α-Rha(2→1)

Glc: glucose; Rha: rhamnose; Xyl: xylose; [a], position of linkage.

As Figure 1 illustrates, *Stevia* glycosides have a steviol aglycone backbone, and only vary by the type, position, and number of glycone moieties. Rebaudioside A differs structurally from stevioside in that it has three glucopyranosyl groups attached at C-13 of the steviol aglycone. All steviol glycosides are metabolized by intestinal micoflora *via* hydrolysis to the aglycone steviol.

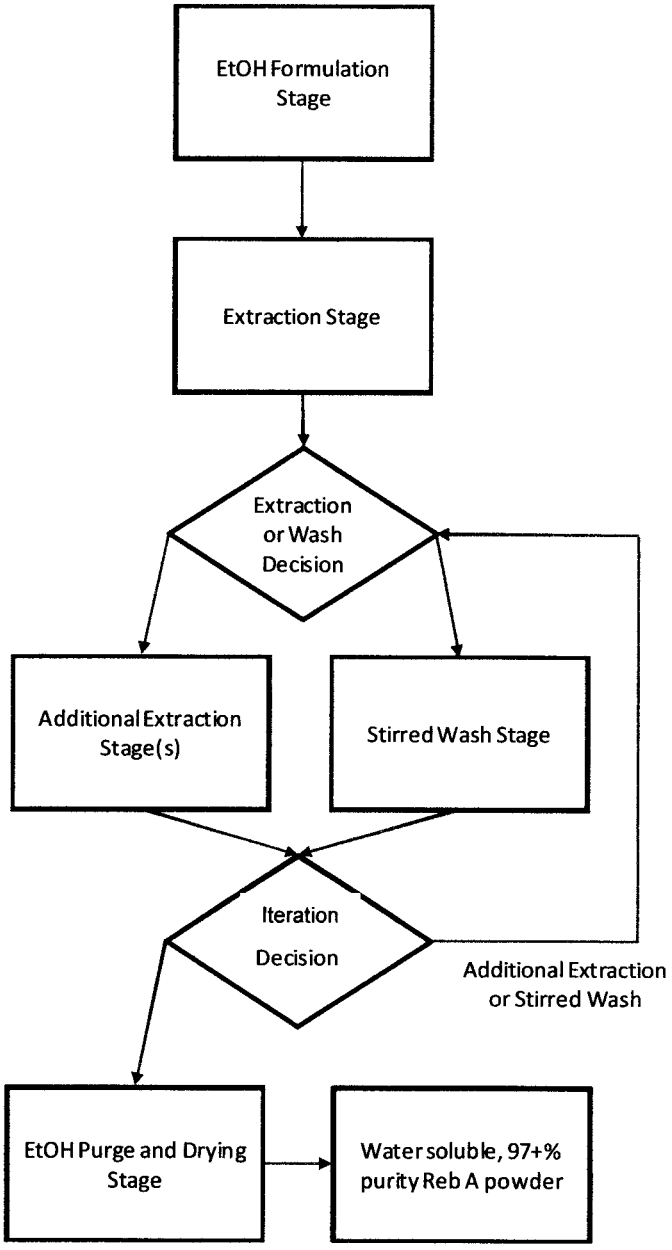
The U.S. Food and Drug Administration (FDA), the Joint FAO/WHO Expert Committee on Food Additives (JECFA), and other regulatory agencies had previously identified gaps in the data supporting the safety of steviol glycosides. Among the issues that required resolution were uncertainties regarding genotoxicity *in vivo* and possible adverse effects on reproductive organs and reproduction. These issues have now been favorably resolved through conduct of additional studies that have now been published, and JECFA has established an acceptable intake (ADI) of 0-4 mg/kg body weight/day for steviol glycosides, expressed as steviol, based on the no-observed-adverse-effect level (NOAEL) for stevioside of 970 mg/kg body weight/day (or 383 mg/kg body weight/day expressed as steviol) in a two-year study in rats and a safety factor of 200 (WHO, 2008). The material evaluated by JECFA contained 95% glycosylated derivatives of steviol, primarily stevioside, rebaudiosides A and C and dulcoside A, with minor amount of rubusoside, steviolbioside, and rebaudiosides B, D, E, and F. Food Standards Australia New Zealand has also established an acceptable daily intake (ADI) of 4 mg/kg body weight/day for steviol glycosides, expressed as steviol (FSANZ, 2008).

B. Manufacturing

The flow diagram shown in Figure 2. provides an overview of the Rebiana manufacturing process. The basic GMP-compliant process involves three stages: (1) ethanol (ETOH)

formulation; (2) extraction; and (3) ethanol purge and drying. As illustrated in Figure 2, additional extraction and/or stirred wash steps may be employed as necessary to maximize the purity of the final product.

Figure 2. Overview of the Rebiana manufacturing process



C. Product Specifications

Table 1 provides a summary of the specifications for *Stevia*-derived Rebiana manufactured by Sweet Green Fields, LLC. Table 2 lists the established limits for microbiological content.

Table 1. Specifications for Rebiana

Specification Parameter	Specification	Analytical Method
Chemical Specifications		
Rebaudioside A	97% minimum (dry weight basis)	HPLC (dry weight)
Other related steviol glycosides[a]	3% maximum (dry weight basis)	HPLC (dry weight)
Moisture	< 6.0% (loss on drying, 105 °C, 2 hr)	FCC Vol. 5
Ash	< 0.2%	FCC Vol. 5
pH	4.5-7.0 (1% w/v in water)	FCC Vol. 5
Solubility	Soluble up to 40% (w/v) in water at 25 °C	FCC Vol. 5
Physical Specifications		
Appearance	White to off white powder	Visual inspection
Odor	None	Olfactory
Taste	Sweet	Taste
Heavy Metals Specifications		
Arsenic	≤ 1 ppm	EPA SW6010B
Barium	≤ 1 ppm	EPA SW6010B
Cadmium	≤ 1 ppm	EPA SW6010B
Chromium	≤ 1 ppm	EPA SW6010B
Lead	≤ 1 ppm	EPA SW6010B
Selenium	≤ 1 ppm	EPA SW6010B
Silver	≤ 1 ppm	EPA SW6010B
Mercury	≤ 1 ppm	EPA SW7471
Other Solvent Residues		
Ethanol	≤ 0.5% (w/w)	FCC Vol. 5
Methanol	≤ 0.02% (w/w)	FCC Vol. 5

EPA: U.S. Environmental Protection Agency
FCC: Food and Chemical Codex
FDA: U.S. Food and Drug Administration
HPLC = high performance liquid chromatography

Table 2. Established microbiological limits for Rebiana

Microorganism	Established Limit
Total Plate Count (CFU/g)	$< 10^4$
Yeast	$< 10^3$
Mold	$< 10^3$
Coliform	Negative
E. coli	Negative
Salmonella	Negative
Staphylococcus	Negative
Listeria	Negative

CFU: colony-forming units

D. Quality Control

Each lot of Rebiana is tested for compliance with the established specifications and microbiological limits. The results of analysis of three non-consecutive lots of Rebiana manufactured by Sweet Green Fields are summarized in Tables 3a and 3b; certificates of analysis are attached as Appendix 1. All analytical data show compliance with the established specifications.

Table 3a. Results of Rebiana batch analyses

Specification Parameter	Specification	Manufacturing lot number and date		
		SGF08006 July 2008	SGF08009 July 2008	SGF08012 July 2008
Rebaudioside A (%)	≥ 97	Conforms	Conforms	Conforms
Other related steviol glycosides (%)	≤ 3	Conforms	Conforms	Conforms
Moisture (%)	< 6.0	Conforms	Conforms	Conforms
Ash (%)	< 0.2	Conforms	Conforms	Conforms
pH	4.5-7.0	Conforms	Conforms	Conforms
Solubility	Soluble up to 40% (w/v) in water at 25 °C	Conforms	Conforms	Conforms
Appearance	White to off white powder	Conforms	Conforms	Conforms
Odor	None	Conforms	Conforms	Conforms
Taste	Sweet	Conforms	Conforms	Conforms
Ethanol residue (% w/w)	< 0.5	Conforms	Conforms	Conforms

Table 3b. Results of microbiological analysis of three Rebiana batches

Microorganism	Established Limit	Limit of Quantification (LOQ)	Manufacturing lot number and date		
			SGF08006 July 2008	SGF08009 July 2008	SGF08012 July 2008
Total Plate Count (CFU/g)	$< 10^5$	100	< LOQ	< LOQ	< LOQ
Yeast	$< 10^4$	100	< LOQ	< LOQ	< LOQ
Mold	$< 10^4$	10	< LOQ	< LOQ	< LOQ
Coliform	Negative	3	< LOQ	< LOQ	< LOQ
E. coli	Negative	3	< LOQ	< LOQ	< LOQ
Salmonella	Negative	< LOQ	Negative	Negative	Negative
Staphylococcus	Negative	3	< LOQ	< LOQ	< LOQ
Listeria	Negative	< LOQ	Negative	Negative	Negative

III. Intended Use and Dietary Exposure

Sweet Green Fields, LLC intends to supply Rebiana for use as a food ingredient in the United States. Rebiana (rebaudioside A, 97% purity) would be used as a sweetener in a variety of food products, including cereals and energy bars, and diet soft drinks, fruit juice drinks, and iced teas at levels consistent with the ADI of 0-4 mg/kg body weight/day (as steviol molar equivalents or, based on molecular weight, one-third of rebaudioside A value) established by JECFA for steviol glycosides (WHO, 2008).

Using dietary exposure data for sweetener use in various countries, Renwick (2008a) performed an intake assessment of rebaudioside A shows the overall projected intakes by average and high consumers in different groups (Table 4).

Table 4. Projected dietary exposures (mg/kg body weight/day) to rebaudioside A based on sweetener substitution (Renwick, 2008a)

Population group	Intakes of intense sweeteners[1]		Projected intakes of rebaudioside A		Steviol equivalents[2]	
	Average consumer	High consumer	Average consumer	High consumer	Average consumer	High consumer
Non-diabetic adults	255	675	1.3	3.4	0.4	1.1
Diabetic adults	280	897	1.4	4.5	0.5	1.5
Non-diabetic children	425	990	2.1	5.0	0.7	1.7
Diabetic children	672	908	3.4	4.5	1.1	1.5

[1] Expressed as sucrose equivalents.
[2] Values are estimated as one-third of rebaudioside A values since the molecular weight of rebaudioside A is about three times that of steviol (*i.e.*, 967 *vs.* 318).

The projected rebaudioside A exposures are based on sweetener substitution (expressed as sucrose equivalents in mg/kg body weight/day) and assume a relative sweetness for rebaudioside A that is 200 times that of sucrose. In the general non-diabetic adult population, the predicted

rebaudioside A exposure from consumption of foods containing rebiana (rebaudioside A, 97%) is 1.3 mg/kg body weight/day for average consumers and 3.4 mg/kg body weight/day for high consumers. Children and individuals with diabetes would be expected to have the highest exposures. As shown in Table 4, when expressed as steviol, these intakes are below the ADI established by JECFA for steviol glycosides (WHO, 2008).

Sweet Green Fields, LLC will notify each food manufacturer purchasing Rebiana that the use of this ingredient in foods is generally recognized as safe (GRAS), provided consumption of such foods does not result in daily intakes exceeding 4 mg steviol equivalents per kg body weight per person, as established by JECFA (2008).

IV. Safety of Rebiana

A. Historical Background

The safety of steviol glycosides has been evaluated by various national and international authorities, including the U.S. Food and Drug Administration (FDA, 2007), the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2008), and Food Standards Australia New Zealand (FSANZ, 2008). JECFA's first evaluation (of stevioside) took place at its 51st meeting in 1998; additional reviews were made at subsequent meetings (WHO 2006; 2008). However, due to gaps in the toxicological database, the Committee was unable to recommend an acceptable daily intake (ADI) until the 63rd meeting in 2004 (WHO, 2006), when a temporary ADI of 0-2 mg/kg body weight/day, expressed as the aglycone steviol, was established. Information supplied to the 68th JECFA meeting (JECFA, 2008) allowed the establishment of final specifications, and the deficiencies identified earlier were resolved, along with a few other debated safety issues. These issues are outlined and addressed subsequently.

1. Specifications

Among the elements found by JECFA to be lacking were precise material specifications, describing the levels of all steviol glycosides, methods of analysis, residual solvents, purity, and a validated method of manufacture (under current Good Manufacturing Practices). Early studies of steviol glycosides employed crude and/or poorly-characterized *Stevia* extracts. However, more recent studies have employed highly purified (>90%) and/or standardized steviol glycosides (SCF, 1999a; 1999b; Carakostas *et al.*, 2008; Prakash *et al.*, 2008).

JECFA finalized the specifications for steviol glycosides during the recent 69th meeting in June, 2008. Steviol glycosides are defined in the specifications as a product obtained through aqueous extraction of the leaves of *Stevia rebaudiana* Bertoni that is subjected to resin absorption and solvent extraction to yield a purified product with stevioside and rebaudioside A as the principal components. The final product must contain at least 95% steviol glycosides (stevioside, rebaudioside A, rebaudioside C, dulcoside A, rubusoside, steviolbioside, or rebaudioside B) on a dried basis (JECFA, 2008). These revised specifications will be considered by the Codex Alimentarius Commission at the 41st Session in March, 2009.

2. Metabolism

Absorption, distribution, metabolism, and excretion (ADME) studies of steviol glycosides have been reviewed and provided the necessary data base for interpreting the safety assessment studies needed to establish the ADI (WHO, 2006; Carakostas *et al.* 2008; Renwick and Tarka, 2008). All steviol glycosides are metabolized (hydrolyzed) *in vivo* to steviol (SCF, 1999a; Renwick and Tarka, 2008; Roberts and Renwick, 2008).

Early studies provided information about the fate of steviol glycosides in rats, but human data were limited. In the rat, steviol glycosides were shown to be poorly absorbed from the upper gastrointestinal tract. Absorption occurred in the lower intestine, but only after cleavage of glycoside units through hydrolysis by intestinal microflora, *i.e.*, as the aglycone steviol (Renwick, 2006; 2008a; 2008b; Renwick and Tarka, 2008; Roberts and Renwick, 2008). More recent studies have shown that the metabolism of steviol glycosides by humans is similar, although some differences in the primary route of excretion (feces in rats *vs.* urine in humans) have been identified (Geuns *et al.*, 2006; 2007a). More detailed discussions about the ADME of

steviol glycosides appear in subsequent sections of this notification. In summary, the available data indicate that stevioside and rebaudioside A, the main glycosides in the *Stevia rebaudiana* plant, are metabolized to steviol in a similar manner in both rats and humans. This finding justifies the use of toxicological safety studies employing stevioside to assess the safety of rebaudioside A (Rebiana).

3. Effects on Blood Pressure and Glucose Homeostasis

Following review of additional safety studies, JECFA raised questions about the potential of chronic steviol glycoside exposure to adversely affect hemodynamic parameters in normo- or hypotensive humans and to affect glucose homeostasis in individuals with diabetes. These questions have now been addressed through the conduct of multiple human studies, which showed no adverse effects on blood pressure (Ferri *et al.*, 2006; Barriocanal *et al.*, 2008; Maki *et al.*, 2008a) or glucose homeostasis (Jeppesen *et al.*, 2006; Maki *et al.*, 2008b). These studies are discussed in more detail in subsequent sections of the present notification.

4. Reproduction and Offspring Development

Older published studies employing crude *Stevia* extracts showed possible effects on the reproduction and/or fertility of rats. Mazzei-Planas and Kuc (1968), for example, observed reduced fertility in female rats exposed to approximately 0.5 g dried plant material per day *via* drinking water prior to and throughout mating. In male rats, exposure to 1.33 g dried leaves/2 ml orally *via* gavage up to twice per day for 60 days was associated with lower plasma testosterone levels and lower testis, epididymis, seminal vesicle weights, and lower spermatozoa concentrations (Oliveira-Filho *et al.*, 1989; Melis, 1999). However, no histopathological findings

were observed. These findings in rats, coupled with anecdotal information that stevia has been used as an oral contraceptive by women from Paraguayan Matto Grosso Indian tribes (Kinghorn, 2002), prompted further experimental reproductive safety investigations. Thus, more recent studies in rats (Mori *et al.*, 1981; Usami *et al.*, 1995) and hamsters (Yodyingyuad and Bunyawong, 1991) with purified extracts were conducted, and are discussed in more detail elsewhere in the present notification. These studies have not shown any evidence of adverse effects on reproduction or offspring development.

5. Genotoxicity

Whereas stevioside and rebaudioside A have not shown evidence of genotoxicity *in vitro* or *in vivo* at up to 2000 mg/kg body weight/day (JECFA, 2005), in some early studies, steviol (or its metabolites) exhibited potential genotoxic activity *in vitro*. However, as discussed elsewhere in this notification, questions regarding the reliability of these early findings have been raised (Geuns, 2007b; Williams, 2007; Brusick, 2008). More recent *in vivo* studies showed no DNA damage or micronucleus formation in rats (Temcharoen *et al.*, 2000), mice (Sekihashi *et al.*, 2002), or hamsters (Temcharoen *et al.*, 2000) receiving steviol at doses up to 8,000 mg/kg body weight, indicating that the limited genotoxicity seen *in vitro*, was due to experimental error (Geuns, 2007b; Williams 2007) and is not expressed *in vivo* (Brusick, 2008).

6. Carcinogenicity

In multiple studies, discussed further in subsequent sections, stevioside showed no evidence of carcinogenicity in male or female rats of Wistar and Fischer 344 strains following dietary administration at levels up to 5% (up to ~2 g/kg body weight/day) for two years. In Wistar rats,

no compound-related preneoplastic or neoplastic lesions were observed (Xili *et al.* 1992). Similarly, in F344 rats, no increased compound-related neoplastic findings were reported (Toyoda *et al.*, 1997). The negative results of the two rat chronic toxicity/carcinogenicity studies, combined with the results of subchronic toxicity (Curry and Roberts, 2008; Nikiforov and Eapen, 2008) and reproductive safety studies (Curry *et al.*, 2008), and the absence of genotoxic activity support the conclusions by JECFA that steviol glycosides do not pose a safety risk (WHO, 2006).

With the satisfactory resolution of these issues, JECFA established an ADI of 0-4 mg/kg body weight for steviol glycosides, expressed as steviol, at the 69th JECFA meeting in June of 2008. The ADI was based on the no-observed-effect level (NOEL) for stevioside of 970 mg/kg body weight/day (or 383 mg/kg bw/day expressed as steviol) in the second two-year study in rats (F344) and a safety factor of 200 (WHO, 2008). The material evaluated by JECFA contained 95% glycosylated derivatives of steviol, primarily stevioside, rebaudiosides A and C, and dulcoside A, with minor amount of rubusoside, steviolbioside, and rebaudiosides B, D, E, and F. FSANZ (2008) has also established an ADI of 4 mg/kg body weight/day for steviol glycosides, expressed as steviol.

B. Absorption, Distribution, Metabolism, and Excretion (ADME)

For the purposes of this GRAS determination, an understanding of the ADME for the steviol glycosides is necessary to (1) establish that experimental animal toxicity studies on stevioside are relevant for assessing the safety of rebaudioside A for human consumption; and (2) establish that rats and mice are appropriate experimental animal models for studies on steviol glycosides. As such, this section provides a summary of the published pharmacokinetic and metabolic data currently available in the rat (mainly) on steviol glycosides and steviol, and compares them to human data.

Experiments with everted gastrointestinal sacs of rats (Koyama *et al.*, 2003a) and Caco-2 cell layers (Geuns *et al.*, 2003) have shown that the transport of stevioside across intestinal mucosa is low, likely due to its molecular size. In addition, steviol glycosides were reportedly resistant to degradation by human digestive enzymes of the mouth, stomach, and small intestine (Koyama *et al.*, 2003b; Hutapea *et al.*, 1997). On the contrary, *in vitro* and *in vivo* studies demonstrated that both stevioside and rebaudioside A were hydrolyzed to the aglycone steviol by microflora in the colon *via* the successive removal of glucose units (Gardana *et al.*, 2003; Geuns *et al.*, 2003, 2007a), and that this hydrolysis is necessary for absorption of steviol derived from Rebiana. Hydrolysis of steviol glycosides also reportedly occurred following incubation with intestinal microflora from the rat cecum (Wingard *et al.*, 1980) and human fecal bacteria (Gardana *et al.*, 2003; Koyama *et al.*, 2003b).

Renwick and Tarka (2008) reviewed the published data on the role of gut microbiota in the metabolism of stevioside and rebaudioside A in order to establish the relevance of stevioside

toxicological data to the safety assessment of rebaudioside A. As indicated by the authors, Rowland *et al.* (1986) showed that comparable β-glucosidase activity was present in the cecal contents/feces of rats, mice, and humans. This finding was not unexpected since Hawksworth and Hill (1971) had previously reported β-glucosidase activity for most of the major groups of intestinal organisms such as enterococci, lactobacilli, clostridia, bacteroides and bifidobacteria. These organisms represent the major types of bacteria in the gastrointestinal tract of most species, and despite species differences in the levels of bacteria in different parts of the intestine, the levels of enterococci, lactobacilli, bacteroides and bifidobacteria in the large intestine are similar among rats, mice, and humans (Hawksworth and Hill, 1971). Similarly, Tamura *et al.* (1980) demonstrated the existence of similar glycosidase activities towards a number of xenobiotic glycosides occurring in enzyme preparations from human feces (fecalase) and rat cecum (cecalase). According to Renwick and Tarka (2008), the studies of Rowland *et al.* (1986), Hawksworth and Hill (1971), and Tamura *et al.* (1980) support the conclusion that the basic metabolic activity towards glucosides is similar in rats, mice, and humans, and in turn, data from the two rodent species would be relevant to humans.

Renwick and Tarka (2008) also identified a number of studies examining the hydrolysis of stevioside and rebaudioside A by the human intestinal microbiota (Wingard *et al.*, 1980; Hutapea *et al.*, 1997; Ishii-Iwamoto and Brache, 1995; Gardana *et al.*, 2003; Koyama *et al.*, 2003b). According to the authors, the most comprehensive of these studies was conducted by Gardana *et al.* (2003) who examined the *in vitro* transformation of stevioside and rebaudioside A after incubation with human microflora, the identity of organisms able to metabolize stevioside and rebaudioside A, and the influence of these sweeteners on the human microflora. The experiments

were conducted under strict anaerobic conditions in batch cultures inoculated with mixed fecal bacteria from healthy human volunteers (6 men and 5 women, ages 20 to 50 years). Feces from five subjects were tested with stevioside or glucose and feces from six subjects were tested with rebaudioside A or glucose. Stevioside was completely degraded to its aglycone steviol after 10-hour incubation with human intestinal microflora. Hydrolysis progressed through formation of steviolbioside, with its concentration peaking after 2-4 hours of incubation, and then decreasing rapidly to zero. After 3-4 hours of incubation, steviol was detected and its concentration increased rapidly thereafter. According to the authors, the initial hydrolysis to steviolbioside suggests that the *j3* 1:19 bond is hydrolyzed more rapidly than the α 1:13 linkage.

Rebaudioside A was also completely metabolized to steviol by human microflora, although, 24 hours was required. Following an initial lag phase of 6-7 hours, steviolbioside was detected. It reached a peak concentration at 12-15 hours, and then was rapidly converted to steviol. The final microbial metabolite of stevioside and rebaudioside A, steviol, remained unchanged during 72-hour incubation with human microflora, suggesting that bacterial enzymes are unable to cleave the steviol structure.

In order to evaluate which microbial groups were involved in the metabolism of stevioside and rebaudioside A, Gardana *et al.* (2003) separated bacterial colonies grown on different selective media and suspended them in incubation medium with added stevioside and rebaudioside A (1 mg/ml). Following 24 and 48 hours of anaerobic incubation, the cultures of selected microbial groups (*i.e.*, bacteroides, bifidobacteria, clostridia, coliforms, enterococci, and lactobacilli) were analyzed by liquid chromatography/diode array detection mass spectrometry (LC-DAD-MS) to

monitor the biotransformation of stevioside and rebaudioside A. Only the most predominant of the intestinal bacteria, the bacteroides, hydrolyzed the glycosides and the magnitudes of hydrolysis of stevioside and rebaudioside A were similar under the incubation conditions. Consequently, Renwick and Tarka (2008) suggested that, for humans, limited inter-subject variation in hydrolysis would be expected *in vivo*, since bacteroides greatly outnumber all other bacterial species occurring in the human bowel.

Based on their review of the extensive database on the microbial hydrolysis of stevioside and rebaudioside A, Renwick and Tarka (2008) concluded that the steviol glycosides are not absorbed intact but rather undergo hydrolysis by the intestinal microflora to steviol. In addition, the authors reported that the rate of hydrolysis of stevioside was slightly greater than that of rebaudioside A (Wingard *et al.*, 1980; Koyama *et al.*, 2003b), and that hydrolysis proceeds *via* initial formation of steviolbioside with steviol as the final product of hydrolysis.

The comparative pharmacokinetics and metabolism of steviol derived from stevioside and rebaudioside A were investigated in a randomized, double-blind, crossover study in order to determine whether the two steviol glycosides were metabolized similarly in humans (Wheeler *et al.*, 2008). Healthy adult male subjects (n=8) received single oral doses of rebaudioside A (5 mg/kg) or stevioside (4.2 mg/kg), providing exposure of approximately 1.6 mg/kg of steviol equivalents. Subjects received rebaudioside A or stevioside as an aqueous solution in a randomized sequence with at least 14 days between treatments. Subjects resided in the clinic from the morning prior to dosing, until 72 hours post-dose for each treatment. The results of pharmacokinetic analysis showed that: (1) steviol glucuronide appeared in the plasma of all

subjects following administration of rebaudioside A or stevioside, with median t_{max} values of 12 and 8 hours post-dose, respectively; (2) steviol glucuronide was eliminated from the plasma, with similar $t_{1/2}$ values of approximately 14 hours for both compounds; (3) the steviol glucuronide geometric mean C_{max} value following administration of rebaudioside A was 1472 ng/ml, compared to 1886 ng/ml after administration of stevioside; (4) the geometric mean AUC_{0-t} value for steviol glucuronide after administration of rebaudioside A was 30,788 ng h/ml, compared to 34,090 ng h/ml after administration of stevioside; (5) steviol glucuronide was excreted predominantly in the urine of subjects during the 72-hour collection period, accounting for 59% and 62% of the rebaudioside A and stevioside doses, respectively; and (6) no steviol glucuronide was detected in feces. Based on these results Wheeler *et al.* (2008) concluded that rebaudioside A and stevioside underwent similar metabolic and elimination pathways in humans, with steviol glucuronide excreted primarily in the urine and steviol in the feces. Furthermore, there was no evidence of any effects on safety parameters as determined by clinical laboratory evaluations, vital sign measurements, physical examinations, and reporting of adverse events.

In another recent publication, Roberts and Renwick (2008) describe the comparative pharmacokinetics and metabolism of ^{14}C-radiolabeled rebaudioside A, stevioside, and steviol in Sprague-Dawley (SD) rats. Rebaudioside A was administered orally by gavage at 5 mg/kg body weight; stevioside and steviol were administered 4.2 and 1.6 mg/kg body weight, respectively, equivalent on a molar basis to the rebaudioside A dose. The results of three separate experiments indicated that, in the rat: (1) elimination of radioactivity from plasma was complete within approximately 72 hours; (2) glycoside units were cleaved from rebaudioside A and stevioside in the intestine (through microbial hydrolysis) to produce the aglycone steviol; (3) there was an

inverse relationship between the number of glycoside units and absorption, *i.e.*, steviol was most rapidly absorbed (peak plasma concentration at ~0.5 hours *vs.* 8 hours for rebaudioside A and 4 hours for stevioside); (4) the primary metabolite was steviol, followed by steviol glucuronide(s), and low levels of one or two other metabolites; (5) data from bile duct-cannulated rats showed absorption of approximately 80% of dose and elimination in the bile as steviol glucuronide(s), whereas data from intact rats indicated elimination in the feces primarily as steviol, suggesting there is de-conjugation in the lower intestine of rats. According to the authors, these results indicate that rebaudioside A and stevioside have similar metabolic profiles in rats and humans, supporting the use of toxicological safety studies conducted with stevioside for the safety assessment of rebaudioside A.

Based on all reviewed data, steviol glycoside metabolism is similar in rats and humans. The only difference is that the rat excretes steviol glucuronide primarily in the feces *via* the biliary tract, whereas humans predominantly excrete it in the urine (Guens et al, 2006; Renwick, 2008b). This is due to different molecular weight (mw) thresholds for human and rat biliary excretion of organic anions (Kwon, 2002). In the rat, anions with less than 325 mw and in humans less than 500-600 mw are excreted in urine (Renwick, 2008b). Thus, both rats and humans have very little systemic exposure to steviol glycosides.

C. Nonclinical Safety

1. Single-dose Oral Toxicity

No published single-dose toxicity studies of rebiana (rebaudioside A, 97% purity) were found. However, Toskulkao *et al.* (1997) examined the effect of stevioside and steviol when administered as a single oral (gavage) dose to hamsters, mice, and rats. Details pertaining to the design, conduct, and findings of this study are discussed below and are also provided in Table 5.

The doses used in the Toskulkao *et al.* (1997) study (up to 15 g/kg body weight) far exceeded the 5 g per kg body weight dose that is generally considered the practical upper limit (limit dose) for the amount of a test material that can be administered in one oral gavage dose to a rodent (FDA, 1997; 2000). At 15 g/kg body weight, stevioside (96% purity) was not lethal in Wistar rats, Swiss albino mice, or Syrian golden hamsters (*i.e.*, $LD_{50} > 15$ g/kg body weight) and did not induce any histopathological changes in the liver or kidneys, giving the compound the toxicity rating of "relatively harmless."

Steviol was not lethal to rats or mice at 14 g/kg body weight or lower. At 15 g/kg body weight, it induced deaths in one male and one female in each group of mice and rats (1/15 animals/group). The authors did not specify a cause of death in these animals. Transient signs of toxicity evident in both rats and mice consisted of decreased activity and difficulty in walking, followed by drowsiness and lethargy, which developed within one hour and resolved in most animals that survived within 24 hours after dosing. Gross and microscopic examination of steviol-dosed rats and mice revealed no changes in the liver or kidneys, giving the compound the toxicity rating of "practically non-toxic."

Mortality and signs of toxicity were evident in hamsters receiving steviol. The observed LD_{50} values were 5.2 g/kg bw for male hamsters and 6.1 g/kg bw for female hamsters. Most deaths occurred within 48 hours after dosing. In hamsters receiving high steviol doses, deaths were seen for up to 7 days. The signs of toxicity included increased feces, drowsiness, weakness, decreased activity, and lethargy, which were noted prior to death. An increase in peritoneal fluid was noted in some animals. Macroscopic observations included congestion of liver and kidneys. Histopathological findings included severe degeneration of the renal proximal tubular cells and vacuolation of periportal hepatocytes. Serum chemistry analysis of a subset of animals receiving 5 g/kg body weight of steviol revealed increases in blood urea nitrogen (BUN), creatinine, and total protein levels from 36-72 hours after dosing to levels that were significantly higher than controls. Acute renal failure was offered by the authors as a possible cause of death in steviol-treated hamsters. Nevertheless, despite the serum chemistry and microscopic findings, the compound toxicity rating is "practically non-toxic".

Table 5. Summary of single-dose oral toxicity studies of *Stevia*-derived compounds

Study Design	Results
Route of exposure: Oral (gavage) **Duration:** Single dose, followed by observation for 14 days **Test material:** Stevioside (96% purity) in distilled water Steviol (90% purity) in corn oil **Parameter measured:** Clinical signs, mortality, LD_{50}, serum chemistry (in subset of male hamsters receiving 5 g/kg body weight of steviol), necropsy, histopathology of liver and kidneys **Dosing:** *Syrian golden hamsters* Stevioside (10/sex/group): 0, 8, 12, 15 g/kg body weight Steviol (60/sex/group): 0, 3, 4, 5, 6, 7, 8 g/kg body weight *Swiss albino mice* Stevioside (10/sex/group): 0, 10, 15 g/kg body weight Steviol (15/sex/group): 0, 12, 14, 15 g/kg body weight *Wistar rats* Stevioside (10/sex/group): 0, 10, 15 g/kg body weight Steviol (15/sex/group): 0, 12, 14, 15 g/kg body weight **Reference:** Toskulkao *et al.* (1997)	Stevioside: No signs of toxicity or deaths were seen in any of the species tested; gross and microscopic examination revealed no changes in liver or kidneys. $LD_{50} > 15$ g/kg body weight Steviol: At 15 g/kg body weight, one male and one female in each group of mice and rats died; no cause of death was given. Transient signs of toxicity evident in both rats and mice included decreased activity and difficulty walking, followed by drowsiness and lethargy; signs developed within 1 hour and resolved within 24 hours after dosing in most animals. Gross and microscopic examination revealed no changes in any of the organs examined. $LD_{50} > 15$ g/kg body weight. LD_{50} values were 5.2 g/kg body weight for male hamsters and 6.1 g/kg body weight for female hamsters. Clinical signs noted in hamsters prior to death included increased feces, drowsiness, weakness, decreased activity, and lethargy; an increase in peritoneal fluid was noted in some animals. Most deaths occurred within 48 hours after dosing; in animals receiving high doses, deaths were seen for up to 7 days. Blood urea nitrogen (BUN), creatinine, and total protein levels in hamsters (5 g/kg body weight) increased from 36-72 hours after dosing to levels that were significantly higher than controls. Histopathological examination revealed severe degeneration of the renal proximal tubular cells and vacuolation in periportal hepatocytes.

2. Multiple-dose Oral Toxicity and Carcinogenicity

Published studies evaluating the multiple-dose oral toxicity of rebaudioside A are summarized in Table 6a. There are no published chronic toxicity or carcinogenicity studies of rebaudioside A. Nevertheless, published chronic studies with stevioside are available. These studies are summarized in Table 6b. In addition, Table 6c describes a study employing a crude aqueous *Stevia* extract.

A 3-month dose range-finding study for the 24-month carcinogenicity study was conducted in male and female Fischer 344 (F344) rats, using dietary doses up to 5% stevioside (~2,500 mg/kg bw/day) of 95.6% purity. This study produced no effects except a slight depression in body weight gain (Aze *et al.*, 1991).

A published 9-month initiation/promotion study for the urinary bladder (UB) carcinogenesis with stevioside administered at 5% (~2,500 mg/kg bw/day) of the diet in male F344 rats is also available (Hagiwara *et al.*, 1984). The study showed that stevioside failed to promote urinary bladder neoplasia initiated by N-nitrosobutyl-N-(-4-hydroxylbutyl)amine, a potent UB carcinogen.

In many of the studies described herein, steviol glycosides in general and stevioside in particular, were administered to rats in the diet at levels equal to or higher than the 5% that is generally considered the practical upper limit for the amount of test material (*i.e.*, limit dose) that can be given to animals without interfering with their nutrition status and caloric needs (FDA, 2000).

Curry and Roberts (2008) examined the effects of Rebaudioside A, 97% purity (Rebiana) in Han Wistar rats following exposure in the diet at levels up to 100,000 ppm (10% of diet) for 4 weeks (dose-range-finding and palatability study) (Table 6a) and up to 50,000 ppm (5%) for 13 weeks (Table 6a). At the highest levels, rebaudioside A intakes were estimated to range from 12,000-14,000 mg/kg body weight/day in the 4-week study segment and 3,000-6,000 mg/kg body weight/day in the 13-week portion of the study. Rebaudioside A was not lethal and had no effect on appearance or behavior. However, lower body weight gains, food consumption, and/or food conversion efficiency (calculated from body weight and food consumption data corrected for caloric density of the diet) were noted at 25,000 ppm (2.5%) and higher (2,000-4,000 mg/kg body weight/day) in both the 4- and 13-week segments; at 12,500 ppm (700-1,500 mg/kg body weight/day), effects on body weight were evident only in males. Thus, the NOAEL was considered to be 50,000 ppm or ~ 4,161 and 4,645 mg/kg bw/day in male and female rats, respectively.

To put into context the effects of rebaudioside A on body weights and body weight gains, Curry and Roberts (2008) considered how this compound might have affected the test diets. Because the compound is not easily digested or absorbed in the gastrointestinal tract (colon), rebaudioside A is considered a substance with little or no nutritional value. Its inclusion in the diet would therefore be expected to affect caloric density and palatability, especially at the highest dose levels. As a result, food consumption and possibly food conversion efficiency would decline, leading to reductions in body weights and body weight gains. Indeed, the authors consider the effects of rebaudioside A on body weights and body weight gains in this chronic rat study to be related to reductions in body weight gains associated with reduced food consumption due to poor

palatability early in the study. Reductions in food consumption, they note, especially during the rapid growth phase of an animal, have been shown to have a delayed effect on overall body weight gains and terminal body weights, often a more pronounced effect than was seen initially. Based on criteria developed by Flamm (2003) that consider the effects of poor palatability of the diet (due to high concentrations of test material) on food consumption and body weight, Curry and Roberts (2008) suggested that the effects of rebaudioside A on body weight gain and/or food consumption seen in this study were not adverse compound-related effects *per se,* but rather the result of poor palatability and increased intestinal bulk. Other effects seen at the highest dietary levels included lower serum total bile acid levels, especially in males, higher mean creatinine and urea levels, and slightly lower urine volume with higher specific gravity. There were also some differences between treated and control animals in slightly lower epididymis weight and lower relative heart, kidney, and adrenal weights in females at the highest levels tested (≥ 5% of diet). In males receiving 10% rebaudioside A (12,000-14,000 mg/kg body weight/day), testes weights were slightly lower than control animals. However, there were no corresponding histopathological findings. Moreover, the authors ascribed the effects of elevated serum total bile acid levels to the large amounts of rebaudioside A metabolites processed by the liver. In addition, they suggest that metabolism of rebaudioside A to steviol in the lower intestine, followed by absorption and glucuronidation in the liver and excretion in the feces, might have altered normal bile acid homeostasis. Since the activity of liver enzymes and liver histopathology were within normal limits, this finding was considered by the authors to be of no biological or toxicological consequence. Finally, because the increases in creatinine and urea concentrations were relatively small and within the laboratory's reference ranges, they were considered by the authors to be indicative of dehydration or osmotic loading of the blood rather than of renal

toxicity, which would also be expected to influence urine volume and specific gravity. More importantly, histopathological examination of the kidneys revealed no changes.

Nikiforov and Eapen (2008) examined the effects of rebaudioside A (99.5% purity) in Sprague-Dawley rats following administration in the diet at 500, 1,000, or 2,000 mg/kg body weight/day for 90 days. Slightly lower mean body weight gains were noted in high-dose males. Clinical pathology, histopathology, and all other parameters measured were unaffected by rebaudioside A treatment (Table 6a).

Two studies exploring the chronic toxicity and/or carcinogenicity of stevioside in F344 (Toyoda et al, 1997) and Wistar (Xili et al, 1992) rats were identified in the published literature. Xili *et al.* (1992) observed no signs of toxicity in a 90-day dose-setting study in which Wistar rats received stevioside (85% purity) at 3% and 5% of the diet (approximately 2,000 and 3,000 mg/kg body weight/day, respectively). No signs of toxicity or abnormal behavior and no effects on body weight gain or food conversion efficiency (calculated from body weight and food consumption data and corrected for caloric density in the diet) were noted (Table 6b). In a subsequent chronic toxicity and carcinogenicity segment, with administration of stevioside at 0.2, 0.6, or 1.2% of the diet (128-839 mg/kg body weight/day) for 24 months, no significant differences between stevioside-treated and control animals was present. At 24 months, no signs of toxicity were observed and no significant differences in terminal body weights, food consumption, survival, relative organ weights or tumor incidences were found (Table 6b).

F344 rats receiving stevioside (95.6% purity) at 5% of the diet (2,400 mg/kg body weight/day) for 104 weeks exhibited lower terminal body weights than control animals and a reduced survival rate (60% *vs.* 78% in controls). The mean relative brain weight of females receiving 5% stevioside was significantly lower than that of control females (Table 6b). These effects were not observed at 2.5% stevioside (~1,000 mg/kg body weight/day). With the exception of a lower incidence of mammary adenocarcinoma in females and lower severity of chronic nephropathy in males at all dose levels, stevioside had no effect on tumor incidence (Toyoda *et al.*, 1997).

Finally, Melis (1995) found significantly lower mean arterial pressure (MAP) at 40 and 60 but not at 20 days. At 40 and 60 days the urine flow as a percent of the glomerular filtration rate (V/GFR) and the fractional urinary sodium excretion (FeNa+) were also significantly higher in Wistar rats receiving a crude aqueous extract of *Stevia rebaudiana* leaves (66.7 g of dried leaves/100 ml final solution) daily *via* oral gavage for up to 60 days (Table 6c).

Thus, the weight-of-evidence (WOE) supports the conclusion that stevioside at up to ~8000 mg/kg/d in Wistar or up to ~2000 mg/kg/d in F344 rats did not have any effect on tumor incidence, and as such does not pose a human cancer risk.

Table 6a. Summary of rebaudioside A multiple-dose oral toxicity studies

Study Design	Results
Route of exposure: Diet **Duration:** 4 weeks (dose range-finding palatability study) **Species/Strain:** Rat/HsdBRI Han:Wist **Number of animals:** 10/sex/group **Test material:** Rebaudioside A, 97% purity (common name rebiana) **Dosage** **ppm:** 0 / 25,000 / 50,000 / 75,000 / 100,000 **% of diet:** 0 / 2.5 / 5 / 7.5 / 10 **mg/kg bw/day:** Male *wk 1*: 0 / 3,455 / 6,981 / 10,180 / 14,167 Male *wk 4*: 0 / 2,610 / 5,505 / 8,248 / 11,672 Female *wk 1*: 0 / 3,724 / 7,125 / 10,789 / 14,119 Female *wk 4*: 0 / 2,963 / 6,217 / 9,189 / 13,126 **Parameter measured:** Clinical signs (twice/day), physical exam (weekly), body weight (twice/week), food consumption (daily), clinical pathology, necropsy, microscopic exam of select tissues. **Reference:** Curry and Roberts (2008)	No deaths or effects on appearance or behavior were observed. Terminal body weights and overall (days 0-28) body weight gains were significantly lower in high-dose females. During days 0-4, animals receiving ≥ 50,000 ppm had significantly lower body weight gains. Food consumption was significantly lower at various times in males receiving ≥25,000 ppm and females receiving ≥ 75000 ppm; overall food consumption was significantly lower only in high-dose males. Slight, but statistically significant, effects on clinical pathology included: higher plasma creatinine in females receiving ≥ 75,000 ppm and all males; lower total bile acid and lower urine volume in males given ≥ 75,000 ppm; higher urine specific gravity in males receiving ≥ 75,000 ppm and all females. Necropsy revealed no macroscopic findings. Lower relative heart weights were seen in males at ≥75,000 ppm; higher relative adrenal weights in females at ≥ 50,000 ppm. Testes weights were significantly, lower in 100,000 ppm males, with no corresponding histopathological findings.

Table 6a. Summary of rebaudioside A multiple-dose oral toxicity studies (cont'd)

Study Design	Results
Route of exposure: Diet **Duration:** 13 weeks **Species/Strain:** Rat/HsdRcc Han:Wist **Number of animals:** 20/sex/group **Test material:** Rebaudioside A, 97% purity (common name rebiana) **Dosage** **ppm:** 0 / 12,500 / 25,000 / 50,000 **% of diet:** 0 / 1.25 / 2.5 / 5 **mg/kg bw/day:** Male *wk 1*: 0 / 1,506 / 3,040 / 5,828 Male *wk 13*: 0 / 698 / 1,473 / 3,147 Female *wk 1*: 0 / 1,410 / 2,841 / 5,512 Female *wk 13*: 0 / 980 / 1,914 / 3,704 **Parameters measured:** Clinical signs (twice/day), physical exam (weekly), body weight (1-2 times/week), food consumption (1-2 times/week), sensory reactivity, ophthalmic exams at beginning and end, clinical pathology (subset of animals on days 10, 46, and 89), necropsy, microscopic exam of select tissues. **Reference:** Curry and Roberts (2008)	No deaths or treatment-related effects on appearance, behavior, or sensory reactivity were noted, although there were some sporadic differences in grip strength. There were no differences in ophthalmological findings. Terminal body weights were significantly lower in all treated males compared to controls. In females, terminal body weights were significantly lower only at the highest dose level. Overall (days 1-92) body weight gains were lower in all treated males and in females receiving 25,000 or 50,000 ppm. Sporadic significant reduction in food consumption was seen during the study in high dose males. Serum total bile acid levels were lower in all treated males; this effect was dose-dependent. In females, a similar effect was evident, but the differences did not reach statistical significance. Other statistically significant differences seen in males and females at the end of the study included: higher plasma creatinine in all females and in high-dose males; higher urea in all males and in females receiving ≥ 25,000 ppm; lower bilirubin in high-dose females and in males receiving ≥ 25,000 ppm; lower urine volume in high-dose animals; and higher urine specific gravity in high-dose females.

Table 6a. Summary of rebaudioside A multiple-dose oral toxicity studies (cont'd)

Study Design	Results
Route of exposure: Diet **Duration:** 90 days **Species/Strain:** Rat/Crl:CD(SD) **Number of animals:** 20/sex/group **Test material:** Rebaudioside A, 99.5% purity **Dosage:** 0, 500, 1000, 2000 mg/kg body weight/day **Parameter measured:** Mortality and moribundity (twice/day), clinical signs (once/day), physical exam (weekly), body weights and food consumption (weekly), locomotor activity (subset of animals), ophthalmic exam (weeks -1 and 12), clinical pathology (subset of animals on weeks 2, 5, and 13), necropsy, microscopic exam of select tissues. **Reference:** Nikiforov and Eapen (2008)	Lower mean body weight gains in males receiving 2000 mg/kg body weight/day compared to control; no effects on any other parameter measured.

Table 6b. Summary of stevioside multiple-dose oral toxicity and carcinogenicity studies

Study Design				Results
Route of exposure: Diet (dose-setting study for chronic toxicity and carcinogenicity study) **Duration:** 90 days **Species/Strain:** Rat/Wistar **Number of animals:** 10/sex/group **Test material:** Stevioside, 85% purity				No signs of toxicity or abnormal behavior and no effects on body weight gain or food conversion efficiency were noted.
	Dosage			
ppm:	0	30,000	50,000	
% of diet:	0	3	5	
mg/kg bw/day[1]: (over 98 days)				
Male	0	1,875	3,125	
Female	0	2,100	3,500	
Parameters measured: Clinical signs, body weights, food consumption **Reference:** Xili *et al.* (1992)				

[1] Dosage estimated from food intakes (~20 g/rat/day for males and 17.5 g/rat/day for females) and body weights (~320 g for males and 250 g for females) in Table 2 of Xili *et al.* (1992), which provides data for animals (N=10) during the first 3 months (98 days) of the 13-week study segment.

Table 6b. Summary of stevioside multiple-dose oral toxicity and carcinogenicity studies

Study Design	Results
Route of exposure: Diet **Duration:** 24 months **Species/Strain:** Rat/Wistar **Number of animals:** 45/sex/group **Test material:** Stevioside, 85% purity **Dosage** **ppm:** 0 / 2,000 / 6,000 / 12,000 **% of diet:** 0 / 0.2 / 0.6 / 1.2 **mg/kg bw/day:** (over first 98 days) Male: 0 / 128 / 368 / 749 Female: 0 / 146 / 416 / 839 **Parameters measured:** Clinical signs, body weights, food consumption, clinical pathology, organ weights, necropsy, histopathology **Reference:** Xili *et al.* (1992)	No signs of toxicity were observed. No significant differences were noted between control and treated groups in terminal body weights, food consumption, survival, and in relative organ weights or tumor incidence at 24 months.

Table 6b. Summary of stevioside multiple-dose oral toxicity and carcinogenicity studies

Study Design	Results
Route of exposure: Diet **Duration:** 104 weeks **Species/Strain:** Rat/F344/Du Crj **Number of animals:** 50/sex/group **Test material:** Stevioside, 95.6% purity **Dosage** **ppm:** 0 / 25,000 / 50,000 **% of diet:** 0 / 2.5 / 5 **mg/kg bw/day:** (over entire period) Male: 0 / 969 / 1,997 Female: 0 / 1,120 / 2,387 **Parameters measured:** Clinical signs, body weights, food consumption, hematology, urinalysis, organ weights, necropsy, histopathology **Reference:** Toyoda *et al.* (1997)	Animals receiving 5% stevioside exhibited significantly lower terminal body weights; males in this group also had a lower survival rate than controls (60 *vs.* 78%). With the exception of slightly higher relative brain weights in females receiving 5% stevioside (*vs.* control), there were no significant differences in relative organ weights. The severity of chronic nephropathy was significantly lower in males receiving stevioside. There were no significant differences between treated and control groups in tumor incidence, except for lower incidence of mammary adenoma in females receiving 2.5 and 5%.

Table 6c. Summary of a multiple-dose oral toxicological study employing a crude aqueous *Stevia* extract

Study Design	Results
Route of exposure: Oral (gavage) **Duration:** 20, 40, or 60 days **Species/Strain:** Rat/Wistar **Number of animals:** 10 males/group **Test material:** Crude aqueous extract of *Stevia rebaudiana* leaves **Target dose:** Saline (negative control) or an amount of extract corresponding 66.7 g of dried leaves/100 ml final solution daily **Parameters measured:** Food consumption, growth rate, renal function (glomerular filtration rate and renal plasma flow) and mean arterial pressure (MAP). **Reference:** Melis (1995)	Treatment for 20 days had no significant effects on the measured parameters. After 40 and 60 days, MAP was significantly lower and V/GFR and FeNa+ were significantly higher in animals receiving the crude extract. MAP, mean artieral pressure; V/GFR, urine volume as % of glomerular filtration rate

3. Genetic Toxicity

The genotoxic potential of *Stevia*-derived compounds has been extensively studied, as Tables 7a (for stevioside and rebaudioside A), and 7b (for steviol and steviol derivatives) detail. Following a comprehensive review of these studies, JECFA (WHO, 2006), and more recently Brusick (2008), concluded that rebaudioside A and stevioside are not genotoxic *in vitro* or *in vivo*, despite positive results in some assays.

As shown in Table 7a, all *in vitro* assays measuring the potential of stevioside and rebaudioside A to induce mutations, chromosome alterations, or DNA damage yielded negative results. Stevioside (88.62% purity) was reported to be positive in an *in vivo* test of DNA double-strand breaks (DSBs) in cells of peripheral blood, spleen, liver, and brain of Wistar rats following administration at 4 mg/ml in drinking water for 45 days; DNA damage was seen only after five weeks of exposure (Nunes *et al.*, 2007). However, questions regarding the reliability of this positive *in vivo* assay have been raised by several genetic toxicology experts who have identified a number of methodological and data interpretation problems, including the lack of adequate positive and negative controls (Geuns, 2007b; Williams, 2007; Brusick, 2008). More importantly, if, as the authors suggest, DNA DSBs were due to metabolism of stevioside to steviol or other genotoxic metabolite, such damage would have been evident in blood cells (evaluated weekly) long before week five of the study (Geuns, 2007b; Williams, 2007; Brusick, 2008).

Steviol and its derivatives yielded positive results in a few *in vitro* bacterial mutagenicity assays only after metabolic activation (Pezzuto *et al.*, 1985; Terai *et al.*, 2002) and *in vitro* mammalian

cell gene mutation and chromosome aberration tests, likewise after metabolic activation (Matsui *et al.*, 1996). However, it has been suggested that the mutagenic effects of steviol are specific to bacterial strains with unique characteristics that enhance their sensitivity to mutagens, such as the loss of DNA excision repair in *S. typhimurium* strain TM677, a histidine independent revertant of the commonly used strain TA 1535 (Brusick, 2008). The genotoxicity seen in *in vitro* mammalian cell assays might have been the result of excessively high concentrations of the compound and only after metabolic activation (Matsui et al, 1996). Importantly, more recent *in vivo* assays (Matsui *et al.*, 1996; Oh *et al.*, 1999; Temcharoen *et al.*, 2000; Sekihashi *et al.*, 2002) have shown no evidence of DNA damage or micronucleus formation in rats, mice, or hamsters receiving steviol at doses up to 8,000 mg/kg body weight, indicating that the genotoxicity seen *in vitro* is not expressed *in vivo*. Thus, the weight-of-evidence (WOE) supports the conclusion that stevioside and rebaudioside A are not genotoxic and do not pose a genotoxic risk to humans.

Table 7a. Summary of stevioside and rebaudioside A genotoxicity studies

Endpoint	Test System	Material	Purity (%)	Concentration or dose	Result	Reference
In vitro						
Reverse mutation	*S. typhimurium* TA98, TA100	Stevioside	99	50 mg/plate	Negative[a]	Suttajit *et al.* (1993)
Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA102, TA104, TA1535, TA1537	Stevioside	83	5 mg/plate[e] 1 mg/plate[f]	Negative	Matsui *et al.* (1996)
Reverse mutation	*S. typhimurium* TA98 and TA100	Stevioside	96	50 mg/plate	Negative	Klongpanichpak *et al.* (1997)
Forward mutation	*S. typhimurium* TM677	Stevioside	83	10 mg/plate	Negative	Matsui *et al.* (1996)
Forward mutation	*S. typhimurium* TM677	Stevioside	NS	Not specified	Negative[a]	Medon *et al.* (1982)
Forward mutation	*S. typhimurium* TM677	Stevioside	NS	10 mg/plate	Negative[a]	Pezzuto *et al.* (1985)
Gene mutation *(umu)*	*S. typhimurium* TA1535/pSK1002	Stevioside	83	5 mg/plate	Negative[a]	Matsui *et al.* (1996)
Gene mutation	B. *subtilis* H17 *rec*+, M45 *rec*-	Stevioside	83	10 mg/disk	Negative[a]	Matsui *et al.* (1996)
Gene mutation	Mouse lymphoma L5178Y cells, $Tk^{+/-}$ locus	Stevioside	NS	5 mg/ml	Negative[a,b]	Oh *et al.* (1999)
Chromosomal aberration	Chinese hamster lung fibroblasts	Stevioside	83	8 mg/ml[e] 12 mg/ml[f]	Negative	Matsui *et al.* (1996)
Chromosomal aberration	Human lymphocytes	Stevioside	NS	10 mg/ml	Negative	Suttajit *et al.* (1993)
Chromosomal aberration	Chinese hamster lung fibroblasts	Stevioside	85	12 mg/ml	Negative[e]	Ishidate *et al.* (1984)
Chromosomal aberration	CHL/IU Chinese hamster lung fibroblasts	Rebaudioside A	NS	1.2-55 mg/ml	Negative[a]	Nakajima (2000a)

Table 7a. Summary of stevioside and rebaudioside A genotoxicity studies (cont'd)

Endpoint	Test System	Material	Purity (%)	Concentration or Dose	Result	Reference
In vivo						
Mutation	*D. melanogaster* Muller 5 strain	Stevioside	NS	2% in feed	Negative[b]	Kerr *et al.* (1983)
DNA damage (comet assay)	Male BDF1 mouse stomach, colon, liver	*Stevia* extract	Stevioside, 52; rebaudioside A, 22	250-2000 mg/kg	Negative[c]	Sekihashi *et al.* (2002)
DNA damage (comet assay)	Male ddY mouse stomach, colon, liver, kidney, bladder, lung, brain, bone marrow	*Stevia*	NS	2000 mg/kg	Negative[c]	Sasaki *et al.* (2002)
DNA damage (comet assay)	Male Wistar rat peripheral blood, spleen, liver, and brain	Stevioside	88.62	4 mg/ml in drinking water *ad libitum* for 45 days	Positive	Nunes *et al.* (2007)
Micronucleus formation	ddY mouse bone marrow and regenerating liver	Stevioside	NS	62.5-250 mg/kg	Negative[b]	Oh *et al.* (1999)
Micronucleus formation	BDF1 mouse bone marrow	Rebaudioside A	NS	500-2000 mg/kg body weight per day for 2 days	Negative[d]	Nakajima (2000b)

NS, not specified; [a] With and without metabolic activation (source not specified in original monograph); [b] Inadequate detail available; [c] Killed at 3 h and 24 h; [d] Killed 30 h after second administration; [e] Without metabolic activation; [f] With metabolic activation.

Adapted from WHO (2006) and Brusick (2008).

Table 7b. Summary of genotoxicity studies of steviol and steviol derivatives

Endpoint	Test System	Material	Purity (%)	Concentration or Dose	Result	Reference
In vitro						
Reverse mutation	*S. typhimurium* TA98 and TA100	Steviol	NS	20 mg/plate	Negative[a]	Suttajit *et al.* (1993)
Reverse mutation	*S. typhimurium* TA98 and TA100	Steviol	96	2 mg/plate	Negative	Klongpanichpak *et al.* (1997)
Reverse mutation	*S. typhimurium* TA97, TA98, TA100, TA102, TA104, TA1535 and TA1537	Steviol	99	5 mg/plate	Negative[a]	Matsui *et al.* (1996)
Forward mutation	*S. typhimurium* TM677	Steviol	NS	10 mg/plate[h] 0.5-10 mg/plate[f]	Negative Positive	Matsui *et al.* (1996)
Forward mutation	*S. typhimurium* TM677	Steviol	NS	10 mg/plate[h] 10 mg/plate[f]	Negative Positive	Pezzuto *et al.* (1985)
Forward mutation	*S. typhimurium* TM677	Steviol	NS	NS	Positive[f]	Terai *et al.* (2002)
Forward mutation	*S. typhimurium* TM677	Steviol-16, 17-epoxide	NS	NS	Positive[f]	Terai *et al.* (2002)
Forward mutation	*S. typhimurium* TM677	15α –hydroxysteviol	NS	NS	Negative[a]	Terai *et al.* (2002)
Forward mutation	*S. typhimurium* TM677	15-oxo-steviol	NS	NS	Positive[f]	Terai *et al.* (2002)
Forward mutation	*S. typhimurium* TM677	Steviol methylester	NS	NS	Positive[f]	Terai *et al.* (2002)
Forward mutation	*S. typhimurium* TM677	16-oxo-steviol methylester	NS	NS	Negative[a]	Terai *et al.* (2002)
Forward mutation	*S. typhimurium* TM677	13,16-*seco*-13-oxo-steviol methylester	NS	NS	Positive[f]	Terai *et al.* (2002)
Forward mutation	*S. typhimurium* TM677	13,16-*seco*-13α-hydroxy-steviol methylester	NS	NS	Negative[a]	Terai *et al.* (2002)
Forward mutation	*S. typhimurium* TM677	Steviol methylester 8,13-lactone	NS	NS	Positive[e]	Terai *et al.* (2002)
Gene mutation *(umu)*	*S. typhimurium* TA1535/pSK1002	Steviol	99	625-1250 µg/plate[h] 1259-2500 µg/plate[f]	Positive Positive	Matsui *et al.* (1996)
Gene mutation	*B. subtilis* H17 *rec*$^+$, M45 *rec*$^-$	Steviol	99	10 mg/disk	Negative[a]	Matsui *et al.* (1996)

Table 7b. Summary of steviol and steviol derivatives genotoxicity studies (cont'd)

Endpoint	Test System	Material	Purity (%)	Concentration or Dose	Result	Reference
Gene mutation	Chinese hamster lung fibroblasts	Steviol	99	400 µg/ml[f]	Positive	Matsui *et al.* (1996)
Gene mutation	Mouse lymphoma L5178Y cells, $Tk^{+/-}$ locus	Steviol	NS	340 µg/ml	Negative[a, b]	Oh *et al.* (1999)
Chromosomal aberration	Chinese hamster lung fibroblasts	Steviol	NS	0.5 g/ml[h] 1-1.5 mg/ml[f]	Negative Positive	Matsui *et al.* (1996)
Chromosomal aberration	Human lymphocytes	Steviol	NS	0.2 mg/ml	Negative	Suttajit *et al.* (1993)
DNA damage (comet assay)	TK6 and WTK1 cells	Steviol	NS	62.5-500 µg/ml	Negative[a]	Sekihashi *et al.* (2002)
In vivo						
DNA damage (comet assay)	Male DBF1 mouse stomach, colon, liver; male CRJ: CD1 mouse liver, kidney, colon and testes	Steviol	>99	250-2000 mg/kg	Negative[c]	Sekihashi *et al.* (2002)
Micronucleus formation	MS/Ae mice	Steviol	99	1000 mg/kg body weight	Negative	Matsui *et al.* (1996)
Micronucleus formation	Swiss mouse bone marrow	Steviol	About 90	8000 mg/kg	Negative[g]	Temcharoen *et al.* (2000)
Micronucleus formation	Wistar rat bone marrow	Steviol	About 90	8000 mg/kg	Negative[g]	Temcharoen *et al.* (2000)
Micronucleus formation	Syrian golden hamster bone marrow	Steviol	About 90	4000 mg/kg	Negative[g]	Temcharoen *et al.* (2000)
Micronucleus formation	ddY Mouse regenerating liver	Steviol	NS	50-200 mg/kg	Negative[b]	Oh *et al.* (1999)

NS, not specified; [a] With and without metabolic activation (source not specified in original monograph); [b] Inadequate detail available; [c] Killed at 3 and 24 h; [e] The presence of metabolic activation decreased the mutagenicity; [f] With metabolic activation; [g] Killed at 24, 30, 48 and 72 h. Ratio of polychromatic to normochromatic erythrocytes was decreased at later time-point(s) in females;[h] Without metabolic activation

Adapted from WHO (2006) and Brusick (2008).

4. Reproduction and Developmental Toxicity

Studies evaluating the effects of rebaudioside A on reproduction and developmental toxicity are summarized in Table 8a. Tables 8b, and 8c summarize studies employing stevioside, steviol, or other crude *Stevia* extracts.

While some animal studies employing crude *Stevia* extracts, including one study published several decades ago, have reported effects on the reproduction and/or fertility of rats (Mazzei-Planas and Kuc, 1968; Oliveira-Filho *et al.*, 1989; Melis, 1999) (Table 8c), more recent studies with purified extracts such as stevioside and steviol have not shown any adverse reproductive or developmental effects (Mori et al, 1981; Yodyingunad and Bunyawong, 1991; Usami et al, 1995; Wasuntarawat et al, 1998) (Table 8b).

In female rats, administration of a crude *Stevia* extract *via* drinking water (~0.5 g dried plant material/day) from 12 days prior to and throughout mating was associated with a 50-79% reduction in fertility (*vs.* water alone), calculated as the number of litters relative to the total females in each group (Mazzei-Planas and Kuc, 1968). This effect was also noted after a 50- to 60-day recovery period. The authors noted loss of the tail in 11 pups belonging to two different litters, which at age 12-15 days, lost their tails possibly from dry gangrene (Table 8c). It was unclear whether these pups were from the first litter (after treatment) or the second litter (after recovery). There was no visible cause for this effect. In general, this study was not well controlled.

Administration of a crude *Stevia* extract (66.7 g dried leaves in 100 ml saline) to male Wistar rats

given orally via gavage up to twice per day for 60 days resulted in seminal vesicle weight reduction only (Oliveira-Filho et al, 1989). Crude *Stevia* extract(1.33 g dried leaves/2 ml saline) to male Wistar rats given orally *via* gavage up to twice per day for 60 days resulted in lower plasma testosterone levels than control (saline) animals, lower testis, epididymis, and seminal vesicle weights, and lower spermatozoa concentrations. However, there were no histopathological changes in testis, seminal vesicle, prostate, or epididymis (Oliveira-Filho *et al.*, 1989; Melis, 1999) (Table 8c).

Stevioside has been administered orally to rats and golden hamsters orally *via* gavage, or in the diet or drinking water. Despite being an atypical animal species for the study of reproductive and developmental toxicity, the hamster was used because of its apparent increased susceptibility to the effects of steviol (LD_{50} of ~5 g/kg body weight *vs.* >14 g/kg body weight for mice and rats) (Toskulkao *et al.*, 1997).

Male and female rats exposed to 3% stevioside (95.98% purity) in the diet prior to and during mating, and until the 7th day of pregnancy (females) had lower body weights than controls, with no effects on mating performance or fertility; slightly greater fetal body length (5.15 *vs.* 5.10 cm) and a slightly higher degree of sternebrae ossification (5.79 *vs.* 5.42) were seen at this level (Mori *et al.*, 1981) (Table 8b). The toxicological significance of these fetal variations is unclear, since (1) the differences, while statistically significant, were slight; (2) these variation are commonly seen in prenatal toxicity studies; (3) these variations do not constitute adverse effects *per se* (*i.e.*, indicative of advanced rather than delayed development); and (4) inadequate in-house control data base for this variation.

Oral (gavage) administration of stevioside to female rats during days 6-15 of gestation at up to 1,000 mg/kg body weight/day did not adversely affect fertility or induce fetal alterations (Usami *et al.*, 1995) (Table 8b).

No adverse effects on mating performance, fertility, or reproduction were noted in three successive generations (F_0, F_1, F_2) of golden hamsters delivering three litters each after receiving stevioside orally (gavage and drinking water) during pregnancy and lactation (Yodyingyuad and Bunyawong, 1991). Oral (gavage) administration of doses up to 1,000 mg/kg body weight/day of steviol (90% purity) to hamsters during days 6-10 of gestation resulted in signs of maternal toxicity (including muscular weakness, hypoactivity, ataxia, dry mouth/nose, closed eyes, ocular discharge and reduced body weights), and higher maternal and fetal dose-related increase in mortality (death in 1/20, 7/20 and 5/12 dams receiving 500, 750 or 1000 mg/kg/d, respectively). There was renal tubular dilatation with hyaline casts in dams receiving ≥ 500 mg/kg/d (Wasuntarawat *et al.*, 1998).

Curry *et al.* (2008) conducted a two-generation reproductive toxicity study of rebaudioside A (97% purity, common name rebiana) in Wistar rats. In a preliminary dose-setting and palatability segment, 15-week-old dams with litters that were 6-8 days old received the test material at up to 50,000 ppm (5%) of the diet during lactation days 14-21. Rebaudioside A intakes at the highest dose (HD) level ranged from 9.5-11 g/kg body weight/day. After weaning (day 21), pups were assigned to receive the respective test diet until terminal sacrifice on day 35. Compared to control pups, pups exposed to 37,500 ppm (3.75%) or 50,000 ppm rebaudioside A had a

tendency toward lower mean body weights, especially during days 21-24, and lower food consumption. Necropsy findings were limited to enlarged parotid salivary glands in most 50,000 ppm group animals and one 37,500 ppm group animal. No other effects were observed (Table 8a). Thus, 25,000 ppm was considered suitable as the HD for the subsequent two-generation reproductive toxicity study.

The two-generation study segment involved administration of rebaudioside A to male and female rats at up to 25,000 ppm (2.5%) of the diet; at this dose level, rebaudioside A intakes ranged from approximately 2,200 mg/kg body weight/day (males; females during pre-pairing and gestation) to 5,000 mg/kg body weight/day (lactating females). The parental (F_0) generation received test diet at 7,500 (LD), 12,500 (MD) or 25,000 (HD) ppm continuously from 10 weeks prior to mating until the end of lactation (Table 8a). Terminal body weights and macroscopic observations of the F_0 generation were unaffected. Pups from the first generation (F_1) that were selected for continued dosing were weaned to the respective test diet and continued on that diet through mating, gestation, and lactation; F_2 animals were sacrificed on day 30 after birth. No effects on F_0 body weights or macroscopic examination were present. Body weight and/or food consumption values for the F_1 HD males and MD and HD females were significantly lower than controls. The mean relative liver weights were increased only in MD and HD F_1 females. Microscopic examination of F_1 males showed no effects on testicular morphology or spermatogenesis. The HD F_2 generation body weights and food consumption were significantly lower than controls. The relative brain weights were increased in HD F_2 rats, and the relative splenic weights were reduced in HD F_2 males. These relative organ weight changes were not seen consistently across multiple generations and therefore may have been incidental. Thus, there

were no effects on reproduction or offspring development parameters (Curry *et al.*, 2008) (Table 8a).

Based on all available evidence, it can be stated that the weight-of-evidence (WOE) for rebaudioside A at doses up to 2,400 mg/kg bw/day given at appropriate reproductive and developmental time intervals, supports the conclusion that 2,400 mg/kg/day in Wistar rats, or 1,000 mg/kg bw/day in golden hamsters cause no effects on reproduction or offspring development parameters, and as such rebaudioside A does not pose a human reproductive risk.

Table 8a. Summary of rebaudioside A reproductive and developmental toxicity studies

Study Design	Results
Route of exposure: Diet (dose-setting and palatability study) **Species/Strain:** Rat/HsdBrl: Han Wistar **Number of animals:** F_0: six 15-week-old females with 6-8-day-old litters per group F_1:10/sex/group (maximum 2/sex/litter) **Test material:** Rebaudioside A, 97% purity (common name rebiana) **Dosage** **ppm:** 0 / 25,000 / 37,500 / 50,000 **% of diet:** 0 / 2.5 / 3.75 / 5 **mg/kg bw/day[2]:** F_0 *lactation days 14-16*: 0 / 4,711 / 8,021 / 9,484 *lactation days 17-20*: 0 / 6,291 / 10,045 / 11,386 **Treatment duration:** F_0: Days 14-21 of lactation F_1: Weaning (day 21)-day 35 (following potential exposure during lactation) **Parameters measured:** Clinical signs, physical exam, body weights, food consumption (F_1 after weaning), necropsy, microscopic examination of testes from high-dose males. **Reference:** Curry *et al.* (2008)	No effect on F_0 body weights or macroscopic findings. Microscopic exam of F_1 males revealed no effects on testicular morphology or spermatogenesis. There was a trend toward slightly lower mean body weight gains at 50000 ppm (HD) that lasted until terminal sacrifice on day 35 in F_1 animals; the effect was most notable during days 21-24. Food consumption was also marginally lower in HD rats than the controls. The 25,000 ppm dietary concentration was considered suitable as the HD for the subsequent two-generation reproductive toxicity study.

[2] For the preliminary phase of this study, mean rebaudioside A doses in F_1 animals after weaning (days 21-35) were not provided by the authors and could not be calculated in the absence of F_1 generation food consumption data.

Table 8a. Summary of rebaudioside A reproductive and developmental toxicity studies (cont'd)

Study Design	Results
Route of exposure: Diet **Species/Strain:** Rat/HsdRcc: Han Wistar **Number of animals:** F_0: 30/sex/group F_1: 24-25/sex/group **Test material:** Rebaudioside A, 97% purity (common name rebiana)	At age 25 days, body weights in high-dose (HD) F_1 males and mid (MD)- and HD F_1 females were significantly lower than controls; At 25,000 ppm, F_2 generation terminal body weights (age 30 days) were significantly lower than controls. Some relative organ weights showed inconsistent increases (liver and brain) or decreases (spleen). All other parameters were unaffected by treatment.

			Dosage			
	ppm:		0	7,500	12,500	25,000
	% of diet:		0	0.75	1.25	2.5
	mg/kg bw/day:					
F_0	Male		0	586	975	2,048
	Female	*pre-pairing*	0	669	1,115	2,273
		gestation	0	648-713	1,119-1,169	2,263-2,381
		lactation	0	715-1,379	1,204-2,388	2,602-5,019
F_1	Male		0	734	1,250	2,567
	Female	*pre-pairing*	0	798	1,364	2,768
		gestation	0	562-625	911-1,058	2,036-2,212
		lactation	0	976-1,406	1,752-2,394	3,289-4,893

Treatment duration:

F_0: From 10 weeks prior to mating, during mating, through gestation and lactation

F_1: From weaning (following potential exposure during lactation) through mating, gestation, and lactation (males killed after ~17 weeks of treatment; females at 28 days post-partum)

F_2: Up to day 30 after birth (following potential exposure during lactation)

Parameters measured:

F_0: Body weight, food consumption, mating performance, fertility, gestation length, litter parameters, offspring development and survival, necropsy, sperm count, motility, and morphology, and histopathology

F_1: Body weight, food consumption, mating performance, fertility, gestation length, litter parameters, offspring development and survival, necropsy, sperm count, motility, and morphology, and histopathology

F_2: Necropsy, macroscopic examination of select organs in subsets of animals.

Reference: Curry *et al.* (2008)

Table 8b. Summary of stevioside and steviol reproductive and developmental toxicity studies

Study Design	Results
Route of exposure: Diet **Species/Strain:** Rat/JCL: Wistar (6-week-old males and 11-week-old females) **Number of animals:** 22/sex/group **Test material:** Stevioside, 95.98% purity **Dosage** **ppm:** 0 / 1,500 / 7,500 / 30,000 **% of diet:** 0 / 0.15 / 0.75 / 3 **mg/kg bw/day[3]:** Male *days 1-30*: 0 / 125 / 625 / 2,500 Male *days 30-60*: 0 / 88 / 441 / 1,765 Female *pre-pairing*: 0 / 136 / 511-614 / 1,714-2,857 Female *gestation*: 0 / 104-127 / 346-577 / 2,077-2,308 **Treatment duration:** Males: 60 days (prior to and during mating) Females: 14 days prior to and during mating until 7th day of pregnancy **Parameters measured:** Body weights, food/water consumption, clinical signs, mating performance, Caesarean-section findings (corpora lutea, implantation, live/dead fetuses, resorptions), fetal alterations (external, skeletal, visceral) **Reference:** Mori *et al.* (1981)	At 3%, stevioside produced lower body weights starting on day 3-4; in females, this effect was not evident during pregnancy but in males it persisted until day 42; slightly greater fetal body length and slightly higher degree of sternebrae ossification in fetuses from dams receiving 3% stevioside.

[3] Stevioside intakes were not specified by the authors; estimated intake values were calculated from food consumption and body weight graphs (Figures 2,3,4, and 5).

Table 8b. Summary of stevioside and steviol reproductive and developmental toxicity studies (cont'd)

Study Design	Results
Route of exposure: Oral (gavage) **Species/Strain:** Rat/Wistar **Number of animals:** Pregnant females **Test material:** Stevioside, 95.6% purity **Target dose:** 0, 250, 500, 1,000 mg/kg body weight/day **Treatment duration:** Days 6-15 of gestation **Parameters measured:** Body weights, food consumption, Caesarean-section findings (corpora lutea, implantation, live/dead fetuses, resorptions), fetal alterations (external, skeletal, visceral) **Reference:** Usami *et al.* (1995)	There were no significant differences between stevioside-treated and control animals in any of the measured parameters.

Table 8b. Summary of stevioside and steviol reproductive and developmental toxicity studies (cont'd)

Study Design	Results
Route of exposure: Oral—*via* gavage; during late pregnancy (day 15) and lactation, females exposed *via* drinking water **Species/Strain:** Golden hamster **Number of animals:** 10/sex/group **Test material:** *Stevia rebaudiana*-derived material containing 90% stevioside **Dose:** 0, 500, 1,000, 2,500 mg stevioside/kg body weight/day **Treatment duration:** Throughout pregnancy and lactation over three pregnancies in three successive generations (F_0, F_1, F_2); animals sacrificed shortly after weaning of the third litter. **Parameters measured:** Body weights during days 30-120 of age, period of rapid growth (in females, not during pregnancy), mating performance, fertility, gestation length, litter size, and histopathology of reproductive organs **Reference:** Yodyingyuad and Bunyawong (1991)	Slightly higher body weights in F_0 males receiving 500 mg/kg/day compared to controls. All other parameters were unaffected by treatment. Histological examination revealed no abnormalities in reproductive tissues.
Route of exposure: Oral (gavage) **Species/Strain:** Golden Syrian hamster **Number of animals:** 12 females in high-dose group; 20 females/group in others **Test material:** Steviol, 90% purity **Dose:** 0, 250, 500, 750, 1,000 mg/kg body weight/day **Treatment duration:** Days 6-10 of gestation (killed on day 14) **Parameters measured:** Body weights, clinical signs, Caesarean-section findings (corpora lutea, implantation, live/dead fetuses, resorptions), fetal alterations (external, skeletal, visceral), and histopathological examination of maternal kidneys **Reference:** Wasuntarawat *et al.* (1998)	Signs of maternal toxicity was noted within 3-4 days after treatment with ≥500 mg/kg/day. Dose-related increased fetal mortality was present.

Table 8c. Summary of reproductive and developmental toxicity studies employing crude aqueous *Stevia* extracts

Study Design	Results
Route of exposure: Oral (gavage) **Species/Strain:** Rat/Wistar (25-30 days old) **Number of animals:** 10 males/group **Test material:** Crude aqueous *Stevia rebaudiana* extract **Dose:** Saline or dose equivalent to 1.33 g dried leaves (2 ml of 66.7 g dried leaves/100 ml solution) **Treatment duration:** 60 days **Parameters measured:** Body weights, food consumption, and body weight gains, plasma testosterone and luteinizing hormone (LH), blood glucose, concentration of spermatozoa in epididymis, weight and histopathology of reproductive organs and sex accessory glands **Reference:** Melis (1999)	No effects on terminal body weights, food consumption, or weight gains were noted. The relative weight of testis, epididymis, and seminal vesicle was significantly lower than control animals, as was the concentration of spermatozoa. Plasma testosterone levels were significantly lower. Histological and morphometric analysis of testis, seminal vesicle, prostate, and epididymis revealed no signs of impairment.
Route of exposure: Oral (gavage) **Species/Strain:** Rat/Wistar (25-30 days old) **Number of animals:** 30 males/group **Test material:** Crude aqueous *Stevia rebaudiana* extract **Dose:** Saline or 2 ml of solution containing 66.7 g dried leaves/100 ml **Treatment duration:** Twice daily for 60 days **Parameters measured:** Body weights, glucose and thyroxin analysis, organ weights, zinc content of prostate, testis, submandibular salivary gland, and pancreas, water content of testis and prostate **Reference:** Oliveira-Filho *et al.* (1989)	Seminal vesicle relative weights were significantly lower in treated males compared to control. All other parameters were unaffected by treatment.

Table 8c. Summary of reproductive and developmental toxicity studies employing crude aqueous *Stevia* extracts (cont'd)

Study Design	Results
Route of exposure: Oral (drinking fluid) **Species/Strain:** Rat (90-152 days old) **Number of animals:** Experiment 1: 14 females (1st litter after treatment; 2nd after a recovery period of 50-60 days) Experiment 2: 14 females (1st litter before treatment; 2nd after treatment; 3rd after a recovery period of 50-60 days) Experiment 3: 14 females (1st litter before treatment; 2nd after treatment) **Test material:** 5% *Stevia rebaudiana* decoction (~15 g dried plant in 300 ml water) **Dose:** 10 ml/rat/day administered orally by temporarily replacing water bottle with small bottle containing test material **Treatment duration:** From 12 days prior to mating and through mating **Parameters measured:** Fertility (number of pregnant rats, total number of offspring, % fertility) **Reference:** Mazzei-Planas and Kuc (1968)	Fertility was 57-79 % lower in treated animals; this effect was present even after the recovery period. In Experiment 1, 11 pups belonging to two different litters lost their tails at age 12-15 days.

D. Human Studies

Published human studies employing rebaudioside A and stevioside are summarized in Table 9. Using a randomized, double-blind, crossover study design, Wheeler *et al.* (2008) examined the comparative pharmacokinetics and metabolism of rebaudioside A (98.7% purity) and stevioside (97% purity) in healthy adult males (n= 8). Subjects received a single oral dose of 5 mg/kg rebaudioside A or 4.2 mg/kg stevioside (exposure of approximately 1.6 mg/kg of steviol equivalents) with at least 14 days between treatments. Standard meals and drinks were provided at 4, 10, and 24 hours post-dose. Blood samples were collected at screening, pre-dose, and daily throughout the admission period for hematology and clinical chemistry analyses. Plasma analysis showed that both rebaudioside A and stevioside were hydrolyzed to steviol in the colon prior to absorption. Metabolism and elimination pathways were found to be similar for rebaudioside A and stevioside. These results were found to be consistent with those of a comparative analysis of the pharmacokinetic and excretion/mass balance of ^{14}C-rebaudioside A, ^{14}C-stevioside, and ^{14}C-steviol in rats (Roberts and Renwick, 2008). The primary route of elimination following oral administration of both rebaudioside A and stevioside was in urine as steriol glucuronide, accounting for approximately 59% and 62% of the dose respectively, with feces accounting for ~ 5% of the dose in each of both compounds. The mean $AUC_{0\text{-}inf}$ (ng.h/ml) values for rebaudioside A or stevioside were 46197 or 53211, respectively. There was one mild and not compound (stevioside)-related adverse event reported consisting of ecchymosis at the site of venipuncture for obtaining blood to monitor hematology and clinical chemistry parameters. No action was taken and the event resolved. No compound-related adverse events were reported in this study. In general, based on the recent human and rodent toxicological study data with stevioside that

previously obtained data are relevant for assessing the human safety of rebaudioside A (Wheeler et al, 2008).

Stevioside and steviol (steviol was more potent) were shown to stimulate glucose-dependent insulin secretion from pancreas islets in an in vitro mouse model (Jeppesen et al, 2006). However more recently, evaluations have not provided clear evidence in support of these results (Ferri et al, 2006; Jeppesen et al, 2006). It has been therefore hypothesized that rebaudioside A may have hypoglycemic and antihypertensive effects similar to those reported with stevioside consumption. In order to address JECFA's (2005) request for studies in people with diabetes to help define an acceptable intake, 2 human trials were conducted (Maki et al, 2008a; 2008b). The dosage of 1000 mg/kg/day, which corresponds to more than 7 times the mean projected daily intake for adults with diabetes was used (Renwick, 2008a).

Maki *et al.* (2008a) carried out a randomized, double-blind, placebo-controlled investigation of the effects of daily consumption of 1,000 mg of rebaudioside A (97% purity) on the blood pressure and heart rate of 100 healthy individuals (18 to 73 years of age). Study participants were assigned to receive four 250-mg capsules (2 with morning meal and 2 with evening meal) per day of rebaudioside A or placebo for four weeks. Blood pressure (resting, seated systolic/diastolic, mean arterial) and heart rate were monitored, along with serum chemistry, hematology, urinalysis, and adverse events. There were no differences of clinical significance between treatment and placebo groups in blood pressure, heart rate, or clinical pathology with any of the parameters tested.

Maki *et al.* (2008b) conducted a randomized, double-blind, placebo-controlled investigation of the effects of rebaudioside A (97% purity) on glucose homeostasis in men and women with type 2 diabetes. Study participants were assigned to receive four 250-mg capsules (2 with morning meal and 2 with evening meal) per day of rebaudioside A (1,000 mg/day total) or placebo for 16 weeks, while maintaining a stable diet. The baseline and demographic characteristics were not significantly different between the 2 groups, with subjects on diabetes, antihypertensive and dyslipidemic medications. Glycosylated hemoglobin (HbA1c), a measure of chronic glycemic control, fasting glucose, insulin, ALT, AST, GGT, AP, creatinine, C-peptide, hematology, BP, body weight, lipids and urinalysis were monitored and evaluated. There were no significant differences between dosed and placebo groups in glucose homeostasis, body weights, fasting lipids, blood pressure, or urinalysis. The mean levels of alanine transaminase (ALT), gamma-glutamyl transferase (GGT), and percentage of basophils were significantly ($p<0.005$, $p<0.02$, $p>0.029$, respectively) increased slightly from baseline (week -2) in the rebaudioside A group. Nevertheless, although these changes were statistically significant, the mean levels of these values were still well within the normal ranges, suggesting the difference was likely due to random variation and not clinically significant. The results of this trial add to the body literature (Dyskrog et al, 2005; Maki et al, 2008a) attesting that rebaudioside A is well-tolerated and lacks the pharmacological effects on blood pressure and glucose homeostasis that have been reported in some studies of stevioside and *stevia* extracts. The rebaudioside A dose of 1000 mg/day corresponds to more than 7 times the mean estimated daily intake for adults with diabetes (1.4 mg/kg/day) and more than 2 times the mean expected daily intake for high-intake adults with diabetes (4.5 mg/kg bw/d) (Renwick, 2008a). These results strongly indicate that chronic intake

of 1000 mg/d of rebaudioside A was well-tolerated and did not produce hypoglycemia or alter blood pressure in men and women with type 2 diabetes.

Table 9. Human studies employing rebaudioside A and stevioside

Study Type	Test Material	Measured Parameters	Results	Reference
Design: Randomized, double-blind, crossover **Subjects:** 8 healthy adult males	• 5 mg/kg rebaudioside A, 98.7% purity (common name rebiana) • 4.2 mg/kg stevioside, 96.6% purity (~1.6 mg/kg steviol equivalents) A single oral dose of each as an aqueous solution with at least 14 days in between treatments	Body weights, vital signs, 12-lead ECG, serum chemistry, hematology, adverse events Pharmacokinetic endpoints: steviol and steviol glucuronide in plasma, urine, and feces (pre-dose to 72 hours post-dose)	Sporadic out-of-range clinical pathology results were seen in several subjects. None was considered by the authors to be clinically significant or to show treatment-related trends. A shift from low to normal neutrophil levels was seen in two subjects, as was a shift from high to normal lymphocytes (stevioside). Minor fluctuations in heart rate were noted during the observation period, with no apparent treatment-related trends. The primary route of elimination for both treatments was in urine as steviol glucuronide (59% rebaudioside A and 62% stevioside); feces accounted for about 5% of the dose. Only a trace was recovered as steviol in urine.	Wheeler *et al.* (2008)
Design: Randomized, double-blind, placebo-controlled **Subjects:** 50 healthy adults with normal blood pressure	Placebo (microcrystalline cellulose) Rebaudioside A, 97% purity (common name rebiana), 1000 mg orally (four 250-mg capsules, 2 with morning meal and 2 with evening meal) per day for 4 weeks	Blood pressure (resting, seated systolic/diastolic, mean arterial) and heart rate monitoring, serum chemistry, hematology, urinalysis, adverse events	There were no significant differences of clinical significance between treatment and placebo groups in heart rate, blood pressure or clinical pathology.	Maki *et al.* (2008a)
Design: Randomized, double-blind, placebo-controlled **Subjects:** ~60 individuals with type 2 diabetes	Placebo (microcrystalline cellulose) Rebaudioside A, 97% purity (common name rebiana), 1000 mg orally (four 250-mg capsules, 2 with morning meal and 2 with evening meal) per day for 16 weeks	Body weights, glycosylated hemoglobin, fasting glucose, insulin, and C-peptide, total cholesterol and triglycerides, blood pressure, serum chemistry, hematology, and urinalysis, adverse events	There were no significant differences between treatment and placebo groups in glucose homeostasis, body weights, fasting lipids, or blood pressure. The mean levels of alanine transaminase (ALT), gamma-glutamyl transferase (GGT), and percentage of basophils increased significantly, from baseline (week -2) in the rebaudioside A group. Nevertheless, these changes although significant, had the mean levels of their values still well within the normal ranges.	Maki *et al.* (2008b)

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APPENDIX 1

Rebiana Certificates of Analysis

SGF
Sweet Green Fields, LLC

Certificate of Analysis

Scientific Name: Stevia Rebaudiana (extract)
LOT number: SGF08006
Date of Manufacture: July 2008
Specifications:

Appearance:	White powder	
Odor:	None	
Taste:	Sweet	
Moisture Content:	1.52% (loss on drying. 105°C. 2 hours)	
Ash Content:	<0.2%	
Ethanol Content:	<0.5%	
Solubility in water:	Readily soluble up to 40.0% (w/v) in water at 25deg C. (Caution. re-precipitates over time).	
pH:	5.6 (1% w/v) in water	
Total Rebaudioside A:	Specification: 97% minimum(dry weight basis)	**Actual:**97.39%
Other Glycosides:	Specification: 3% maximum(dry weight basis)	**Actual :** 2.40%
Short-Term Temperature Stability:	Stable to temperatures of 150° C (1% degradation of Reb A after 1 hr at 150° C)	
Long term temp stability:	Shelf Stable at temp of 60°C	

Heavy Metals (ICP):	Method	Reporting Limits (ppm)	MDL (ppm)	Actual Test (ppm)
Arsenic	SW6010B	5	0.52	ND
Barium	SW6010B	10	0.030	ND
Cadmium	SW6010B	2	0.020	ND
Chromium	SW6010B	5	0.18	ND
Lead	SW6010B	20	0.11	ND
Selenium	SW6010B	20	0.13	ND
Silver	SW6010B	5	0.029	ND
Mercury	SW7471	0.2	0.018	ND

ND – Not detected at the Method Detection Limit (MDL).

Microbiological analysis:
All microbial analysis undertaken by a certified microbial analysis laboratory. Details provided upon request.

Microbe	*Count**
Total Plate Count (CFU/g).	<100
Yeast:	<100
Mold.	<10
Coliform:	<3
E. Coli:	<3
Salmonella:	Negative
Staphylococcus	<3
Listeria	Negative

- All numbers showing less than (<) gave counts that were below the limit of quantification for the microbe species described

Mel C. Jackson, Ph.D.
Vice President – Science
QA Officer

Date. 8/18/2008

SGF
Sweet Green Fields, LLC

Certificate of Analysis

Scientific Name: Stevia Rebaudiana (extract)
LOT number: SGF08009
Date of Manufacture: July 2008
Specifications:

Appearance:	White powder	
Odor:	None	
Taste:	Sweet	
Moisture Content:	1.21% (loss on drying. 105°C. 2 hours)	
Ash Content:	<0.2%	
Ethanol Content:	<0.5%	
Solubility in water:	Readily soluble up to 40.0% (w/v) in water at 25deg C. (Caution. re-precipitates over time).	
pH:	5.6 (1% w/v) in water	
Total Rebaudioside A:	Specification: 97% minimum(dry weight basis)	**Actual:**97.38%
Other Glycosides:	Specification: 3% maximum(dry weight basis)	**Actual :** 2.44%
Short-Term Temperature Stability:	Stable to temperatures of 150° C (1% degradation of Reb A after 1 hr at 150° C)	
Long term temp stability:	Shelf Stable at temp of 60°C	

Heavy Metals (ICP):	Method	Reporting Limits (ppm)	MDL (ppm)	Actual Test (ppm)
Arsenic	SW6010B	5	0.52	ND
Barium	SW6010B	10	0.030	ND
Cadmium	SW6010B	2	0.020	ND
Chromium	SW6010B	5	0.18	ND
Lead	SW6010B	20	0.11	ND
Selenium	SW6010B	20	0 13	ND
Silver	SW6010B	5	0.029	ND
Mercury	SW7471	0.2	0.018	ND

ND – Not detected at the Method Detection Limit (MDL).

Microbiological analysis:
All microbial analysis undertaken by a certified microbial analysis laboratory. Details provided upon request.

Microbe	*Count**
Total Plate Count (CFU/g):	<100
Yeast:	<100
Mold	<10
Coliform:	<3
E. Coli:	<3
Salmonella:	Negative
Staphylococcus	<3
Listeria	Negative

- All numbers showing less than (<) gave counts that were below the limit of quantification for the microbe species described

Mel C. Jackson. Ph.D.
Vice President – Science
QA Officer

Date: 8/28/2008

SGF
Sweet Green Fields, LLC

Certificate of Analysis

Scientific Name: Stevia Rebaudiana (extract)
LOT number: SGF08012
Date of Manufacture: September 2008

Specifications:

Appearance:	White powder	
Odor:	None	
Taste:	Sweet	
Moisture Content:	1.40% (loss on drying, 105°C, 2 hours)	
Ash Content:	<0.2%	
Ethanol Content:	<0.5%	
Solubility in water:	Readily soluble up to 40.0% (w/v) in water at 25deg C. (Caution, re-precipitates over time).	
pH:	5.6 (1% w/v) in water	
Total Rebaudioside A:	Specification: 97% minimum(dry weight basis)	**Actual:**97.74%
Other Glycosides:	Specification: 3% maximum(dry weight basis)	**Actual :** 2.25%
Short-Term Temperature Stability:	Stable to temperatures of 150° C (1% degradation of Reb A after 1 hr at 150° C)	
Long term temp stability:	Shelf Stable at temp of 60°C	

Heavy Metals (ICP):	Method	Reporting Limits (ppm)	MDL (ppm)	Actual Test (ppm)
Arsenic	SW6010B	5	0.52	ND
Barium	SW6010B	10	0.030	ND
Cadmium	SW6010B	2	0.020	ND
Chromium	SW6010B	5	0.18	ND
Lead	SW6010B	20	0.11	ND
Selenium	SW6010B	20	0.13	ND
Silver	SW6010B	5	0.029	ND
Mercury	SW7471	0.2	0.018	ND

ND – Not detected at the Method Detection Limit (MDL).

Microbiological analysis:
All microbial analysis undertaken by a certified microbial analysis laboratory. Details provided upon request

Microbe	*Count**
Total Plate Count (CFU/g)	<100
Yeast:	<100
Mold:	<10
Coliform:	<3
E. Coli:	<3
Salmonella:	Negative
Staphylococcus	<3
Listeria	Negative

- All numbers showing less than (<) gave counts that were below the limit of quantification for the microbe species described

Mel C Jackson. Ph.D.
Vice President – Science
QA Officer

Date: 10/02/2008

APPENDIX 2

Expert Panel Statement

EXPERT PANEL STATEMENT

Background

Under section 201(s) of the Federal Food, Drug, and Cosmetic Act, a substance is exempt from the definition of food additive and thus, from premarket approval requirement, if its safety is generally recognized by qualified experts. A determination that a particular use of a substance is generally recognized as safe (GRAS) requires both technical evidence of safety and a basis to conclude that this technical evidence of safety is generally known and accepted. This determination may rely on the opinion or recommendation of an authoritative body and/or the opinion of an "expert panel" specifically convened for this purpose.

Sweet Green Fields, LLC (4164 Meridian Street, Suite 304, Bellingham WA 98226, USA) has requested of the undersigned experts, individuals qualified by scientific training and other relevant experience to evaluate the safety of food and food ingredients, a review of the available information supporting the use of Rebiana in foods for the general population as generally recognized as safe (GRAS), based on scientific procedures. Accordingly, a GRAS dossier summarizing published and unpublished information pertaining to the safety of Rebiana was made available to the expert panel.

Substance Identification

Rebiana is a steviol glycoside derived from the *Stevia rebaudiana* Bertoni plant. Steviol glycosides and extracts of *Stevia*, primarily the leaves, have been used as sweeteners for many years in South America and Asia.

Rebiana (rebaudioside A, 97%) is a standardized product derived through GMP-compliant manufacturing methods. Regular testing of production lots for compliance with the established product specifications ensures consistency in product quality.

Proposed Uses

Rebiana will be used as a sweetener in a variety of food products such as cereals and energy bars, and beverages such as diet soft drinks, fruit juice drinks, and iced teas at levels consistent with the ADI of 0-4 mg/kg body weight/day (as steviol equivalents) established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for steviol glycosides. As the supplier, Sweet Green Fields, LLC will notify each food manufacturer purchasing Rebiana that the use of this ingredient in foods is generally recognized as safe (GRAS), provided consumption of such foods does not result in daily intakes exceeding 4 mg of steviol equivalents per kg body weight per person, as established by JECFA.

Estimated Intakes

The projected exposures to rebaudioside A from consumption of foods containing Rebiana are 1.3 and 3.4 mg/kg body weight/day, respectively, for average and high consumers in the general adult population. Children and individuals with diabetes would be expected to have the highest exposures. These values are based on sweetener (sucrose) substitution and assume a relative sweetness for rebaudioside A that is 200 times that of sucrose.

Safety

ADME studies suggest that the major steviol glycosides rebaudioside A and stevioside are metabolized in an identical manner to the aglycone steviol through hydrolysis by intestinal microflora. Consequently, stevioside safety data are considered relevant in assessing the safety of rebaudioside A and *vice versa.*

The U.S. FDA and other authoritative bodies previously identified gaps in the data supporting the safety of steviol glycosides. Among the issues that required resolution were uncertainties regarding genotoxicity *in vivo* and adverse effects on reproductive organs and reproduction. These issues have now been favorably resolved through conduct of additional studies that have now been published.

Expert Panel Opinion

Having independently and critically evaluated the GRAS dossier summarizing published and unpublished information pertaining to the safety of Rebiana, the undersigned members of the expert panel unanimously conclude that the use of Rebiana as a sweetener in foods for the general population as generally recognized as safe (GRAS), based on scientific procedures. The available information indicates that Rebiana produced using GMP-compliant methods to the established food-grade specifications is not harmful when used at levels consistent with the ADI of 0-4 mg/kg body weight/day (as steviol equivalents) established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) for steviol glycosides.

_____ 15 January 2009
Gary M. Williams, MD (Chairman) (date)
Professor of Pathology, NY Medical College

_____ 1/15/09
Gerald Fisher, PhD (date)
Formerly VP of Drug Safety, Wyeth Research

_____ Jan 15, 2009
Xavier Pi-Sunyer, MC (date)
Professor of Medicine, Columbia University

_____ 15 January 2009
Michael J. Iatropoulos, MD, PhD (Rapporteur) (date)
Professor of Pathology, NY Medical College

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SUBMISSION END